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THE AMERICAN REVIEW OF RESPIRATORY DISEASES

Clinical and Laboratory Studies of Tuberculosis and Respiratory Diseases

OFFICIAL JOURNAL OF AMERICAN TRUDEAU SOCIETY
MEDICAL SECTION OF THE NATIONAL TUBERCULOSIS ASSOCIATION

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(Formerly THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES)

ANNUAL MEETING, LOS ANGELES, CALIFORNIA, May 15–20, 1960

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Membership: All physicians and surgeons in the United States, Canada, and other countries who are interested in tuberculosis and other respiratory diseases may apply for membership in the American Trudeau Society. The dues for active membership in the Society are $18 per year, which includes membership in the National Tuberculosis Association, copies of the Society's News Letter, and a subscription to the REVIEW and to the monthly BULLETIN of the National Tuberculosis Association.

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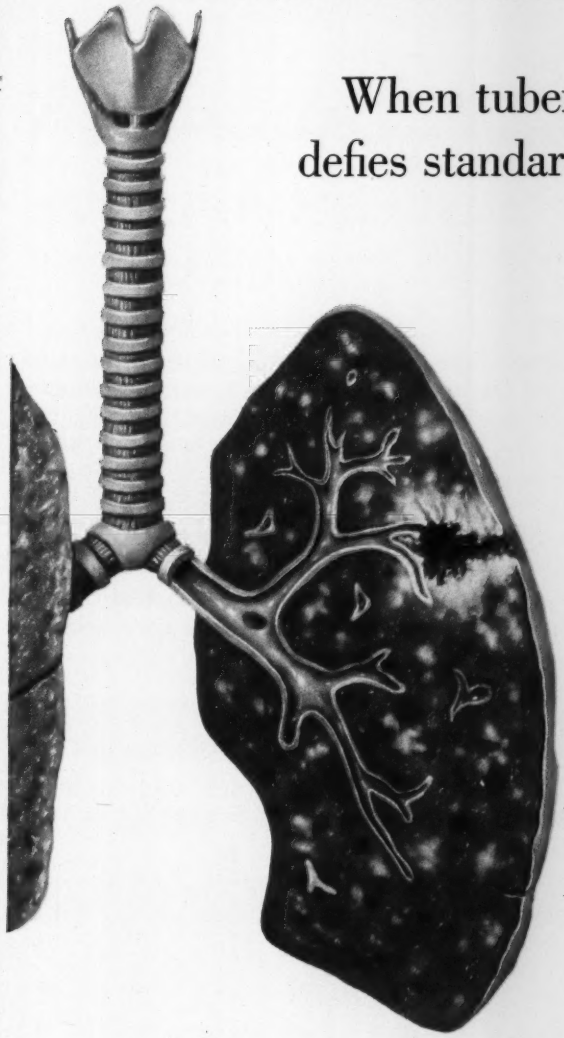

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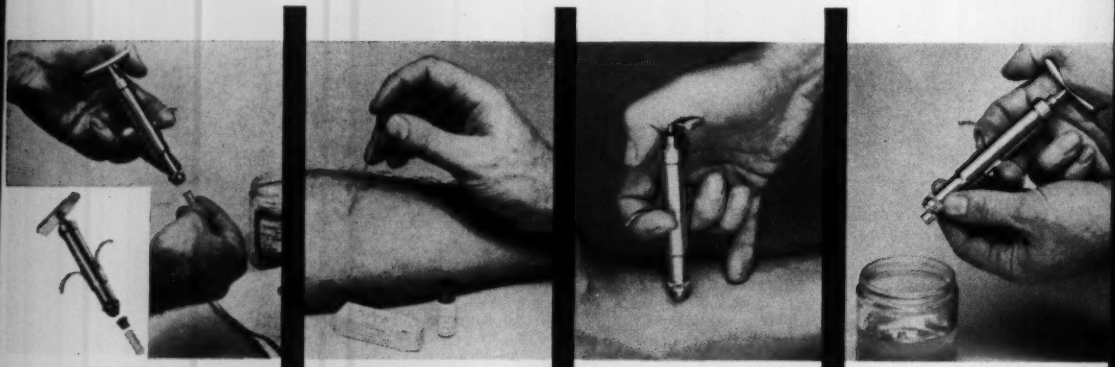

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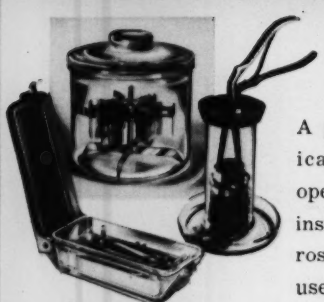

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LOS ANGELES, CALIFORNIA

MAY, 15-20

The 1960 Annual Meeting of the National Tuberculosis Association, the American Trudeau Society, and the National Conference of Tuberculosis Workers will be held in the Statler Hilton and Biltmore hotels, Los Angeles, California, May 15-20.

Registration will be in the Statler Hilton and will begin at 9:00 AM, SUNDAY, MAY 15.

Exhibits will be in the Statler Hilton and will open for viewing at 8:30 AM, MONDAY, MAY 16.

Special interest sessions will begin SUNDAY, MAY 15. *Medical, public health, and nursing sessions* will begin MONDAY, MAY 16.

General sessions will include an opening keynote program Monday at 9:00 AM, followed immediately by the awarding of the Trudeau and Will Ross medals; a session on Monday afternoon at which results of the Arden House Conference on Tuberculosis will be presented (the opening portion will be of special interest to doctors); a session Tuesday morning, and the NTA Business Luncheon on Thursday.

Sleeping rooms will be available in the Statler Hilton, 930 Wilshire Boulevard, Los Angeles 17, and the Biltmore Hotel, 515 South Olive Street, Los Angeles 13.

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Applications for rooms should be made directly to the hotel of choice. In the event the hotel is unable to provide the desired space, requests will be referred to the Los Angeles Convention Bureau for clearing with a cooperating hotel. Confirmation will come direct from hotel accepting reservation.

Room requests must include mention of NTA Annual Meeting and give a definite date and hour of arrival as well as a date of departure. Names and addresses of all persons to occupy the rooms requested must be included.

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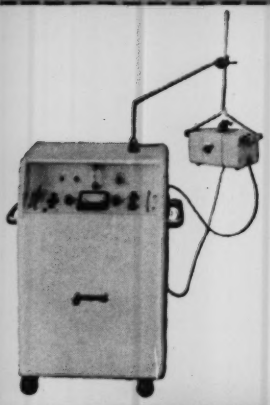

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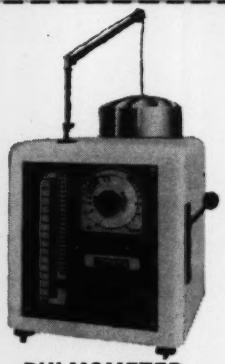

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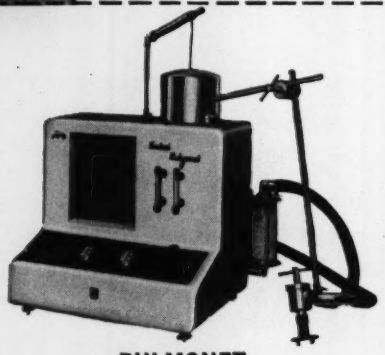

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ERRATUM

The name of the first author in reference (10) of the article entitled "Pneumonectomy in the Treatment of Pulmonary Tuberculosis. The After History" by C. J. Martin and Walter T. Miller, published in the February, 1960, issue of the REVIEW (vol. 81, p. 184), should be *Douglass, R.* instead of Richman, D.

THE AMERICAN REVIEW
OF RESPIRATORY DISEASES
Clinical and Laboratory Studies of Tuberculosis and Respiratory Diseases

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MASS CHEMOPROPHYLAXIS OF TUBERCULOSIS[1]

The Acceptability and Untoward Side Effects of Isoniazid in a Control Study in Greenland

E. GROTH-PETERSEN, ULF GAD, AND F. ØSTERGAARD

(Received for publication January 7, 1960)

INTRODUCTION

In July, 1956, a large-scale control study of the potential value of isoniazid in tuberculosis prophylaxis of adults was started in the general population of Greenland (1). The trial was considered justified because of the tuberculosis problem in Greenland and the world-wide experience of the harmlessness of isoniazid in the treatment of tuberculous patients. The first nationwide tuberculosis survey in West Greenland (1955–1956), which included 93 per cent of the Greenlanders, revealed that practically all adults were infected; the annual incidence of tuberculous disease during the years 1951–1955 was estimated to have been about 2,000 per 100,000 persons (2).

An attempt to use isoniazid for what McDermott (3) has termed *secondary* chemoprophylaxis,[2] that is, to prevent the breakdown of infection into active disease, was inspired by the results of experimental studies (4, 5) as well as by the experience in Denmark and elsewhere (6–8) that the reservoir of latent infections in adults is the main source of new infectious cases. The usefulness of prophylactic isoniazid, however, depends not only on the effect of the drug, but also on whether the population accepts self-administration of the drug. The well-known difficulties in accomplishing individual ambulatory long-term treatment of patients (9–11) might be aggravated when mass medication is prescribed for persons who feel quite well and who, moreover, have just had a roentgenographic examination and been told that they had no signs of tuberculous disease.

The difficulties must be expected to be compounded if the drug causes side effects. Although the toxicity of isoniazid is low, a variety of symptoms have been reported among patients receiving moderate doses of the drug (12, 13). In many cases, however, the causality is doubtful, and the frequency of untoward symptoms in healthy persons is quite unknown.

The Greenland trial offers an opportunity to obtain an estimate of the acceptability of prophylactic medication by a population of presumably healthy adults. Moreover, because the trial has been carried through as a double-blind procedure involving two groups, one group getting isoniazid and the other getting placebo, information may also be obtained on the side effects of isoniazid. The purpose of the present paper is to report observations on the acceptability of the drug and its side effects in the Greenland population; results on tuberculosis morbidity, based on yearly follow-up examinations of the entire population, will be given in later papers.

MATERIAL AND METHODS

The material comprises the entire native population, fourteen years of age and over, of West Greenland, except for those living in the smallest

[1] From the Danish Tuberculosis Index, Copenhagen, Denmark; and Dronning Ingrids Sanatorium, Godthaab, Greenland.

[2] The preferred term is *disease prophylaxis* as distinguished from *infection prophylaxis*.—The Editor.

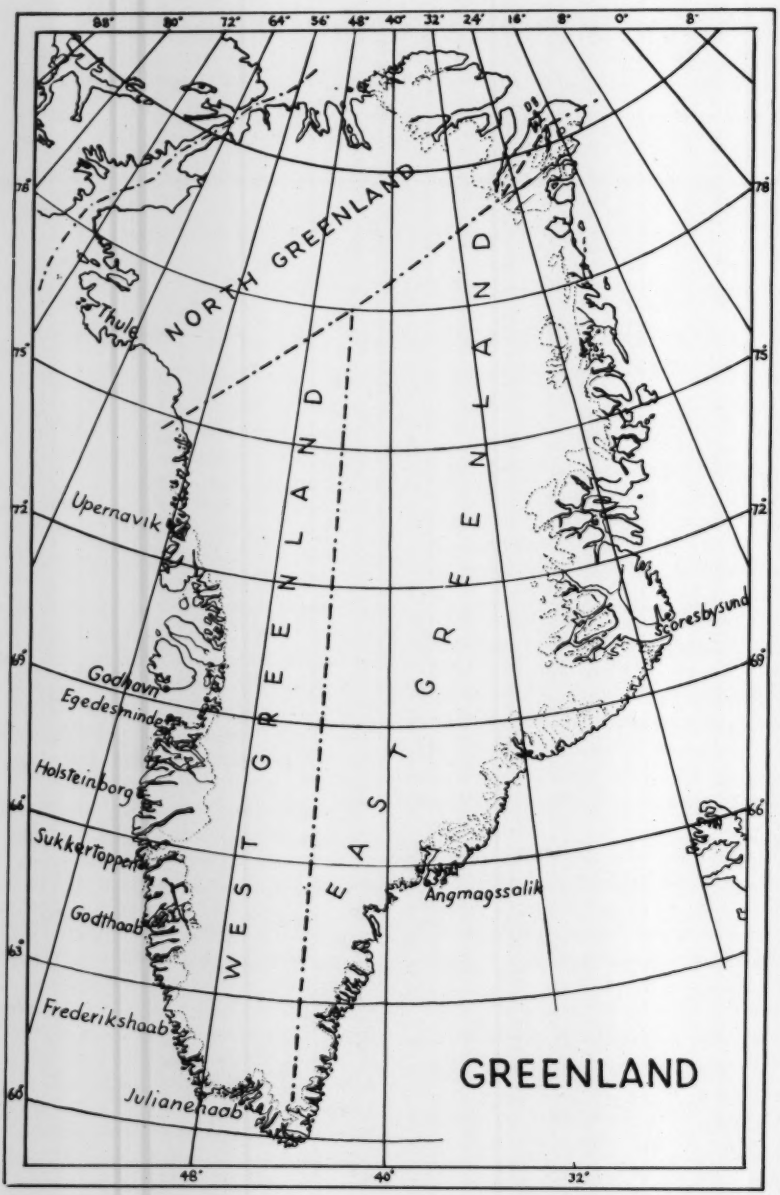

FIG. 1. Map of Greenland, showing major subdivisions, and location of the capital city, Godthaab, in West Greenland.

and most remote villages, which were excluded in advance for administrative and economic reasons.

The people of West Greenland live in villages scattered on islands and peninsulas along the extensive and mountainous coast (figure 1), which extends 60° to 74° north latitude. Measured in a straight line this corresponds to about 1,000 miles, but because of the branching skerries and long fjords the coastline is very much longer. The total population numbers about 24,000 persons, 14,000 of whom are adults and 10,000 are children less than fourteen years of age. The principal occupations are fishing and hunting of sea mammals.

Practically all communication is by sea, as there are no roads outside the towns. In northern districts, where the sea freezes over during the winter, the only communication from December to May is by dog-sled across the ice.

The west coast is divided into 13 medical districts, each having a hospital and a medical officer. Most of the districts also have one or two assistant physicians. Together they serve the population as hospital and family physicians. In addition, there is a 210-bed tuberculosis sanatorium in the capital city, Godthaab. All hospital and medical expenses are paid by the Government.

The 79 villages included in the trial comprised 85 per cent of the adult population of West Greenland; the remaining 15 per cent live in the 54 villages excluded from the trial. Only the adult population was allowed to participate, since, if children had been included, the dose of isoniazid would have had to be varied according to weight or age; and it was considered too risky to try to give different doses to different persons in the same village. It was decided, therefore, to exclude all persons less than fourteen years of age. Moreover, most of the children had been BCG-vaccinated, whereas most persons more than fourteen years of age were naturally tuberculin positive.

As a further condition for participating in the trial, all persons were required to have roentgenographic examinations of the chest. This was provided on board the *Misigssut*, a ship specially equipped with a 70-mm. photofluorographic unit and staffed by a medical team which traveled from village to village along the coast. As described in a previous report (1), the *Misigssut* started its first round in the fall of 1955, calling at each village, where it offered, among other things, roentgenographic examinations of the chest, tuberculin tests, and sputum examinations. About 400 persons were found to have active tuberculosis and were referred for treatment in sanatoriums.

By the fall of 1956, when the *Misigssut* began its second round, plans had been completed for the present control study of the prophylactic use of isoniazid. In view of the very high prevalence of tuberculin reactors in the adult population, tuberculin tests were given in only 5 of the 13 districts; but all persons were examined roentgenographically, as before, the 70-mm. photofluorograms being read independently by two specialists. Sputum examinations were also included as a routine procedure.

Persons with active tuberculosis were referred for treatment and disqualified from participating in the trial. Persons who had been previously treated for tuberculosis were allowed to participate providing their disease was arrested and that all treatment had been completed more than one year earlier. All other adults were invited to participate in the trial after the examinations were completed in each village, the first village starting medication in July, 1956, and the last in November, 1957.

For practical reasons and for publicity purposes, the campaign was named "INA," which is used idiomatically in the Danish language and became a very popular name.

For reasons not relevant to this report, the villages to be included in the trial were first divided into two groups. Within each of these groups, the villages were then arranged according to the number of adult inhabitants; and by use of "random number tables" one of every two successive villages in each group was assigned isoniazid, and the other placebo. It was thus ensured that the number of persons getting isoniazid and placebo would be about the same. The number of adult inhabitants in the villages was the only variable considered in the randomizing process. The doctors in Greenland were informed that the tentative prophylactic isoniazid dose varied, but no one knew that part of the population got only placebo. The dosage and the alternation of villages were known only by two persons, both in Copenhagen; and the result of the randomizing still remains undisclosed so that no one in Greenland (including the medical staff) knows what kind of pills were given in any particular village.

Within each village, all participants got the same kind of pills and similar doses. The dose of isoniazid given to the majority of persons was 400 mg. every 24 hours; a smaller group was given 600 mg. The dose was given in two or three pills a day on two successive days each week. The average dose per kg. of body weight was 7 or 10 mg. per day, the weekly dose thus being only 14 mg. or 20 mg. per kg. No added vitamins were administered.

For simplicity's sake the inefficacious medication for the control group is called placebo, but actually the placebo pills each contained 0.1 mg. of isoniazid. For tactical reasons pure placebo was not given, but an isoniazid dose of 0.1 mg. two or three times a day, two days a week, is regarded as having no pharmacodynamic effect. As is well known, isoniazid has a very bitter taste, perceptible, of course, only if the pills are chewed. Therefore 2 mg. of quinine sulfate were added to the placebo pills to preclude the possibility of distinguishing the pills by taste. The isoniazid and the placebo pills were also the same size, shape, color, and weight.

Both the treatment and control groups were given pills for two periods of thirteen weeks, with

an interval of thirteen weeks between the two treatment periods. Bottles containing the pills were sent from Copenhagen to Greenland, labeled with a special code number for each of the 79 villages.

A highly qualified Greenlander, who served as traveling secretary, was sent out with propaganda material (lectures, printed matter for all the households, et cetera). The traveling secretary and nurses especially appointed for the purpose organized the work with the local authorities, among whom a local medical officer was always included.

In every village a number of trustworthy representatives (usually women) were selected, normally one per twenty households. The nurse distributed the bottles of pills to the representatives, who were then to be in charge of the weekly distribution, as described below.

When the medication had thus started, the traveling secretary left for the next medical district, while the nurse stayed in the district and supervised the work until the end of the medication period. Long and tiresome travel was often required of the nurse to reach the scattered villages within the district. In the northernmost medical district the nurse traveled nearly 900 miles by dog-sled during the winter of 1956–1957.

The local representatives in the villages were supplied with pills and pill boxes for every household and individual record cards for all participants in the project. A member of each household was asked to come to the representative each week to fetch the ration of pills for his household, the date and amount of pills being recorded on the individual cards by the representative.

The procurement of pills each week by members of the household would seem to provide a reasonable check (but no absolute assurance) that the pills were being consumed. In addition, unannounced visits were occasionally made to the homes to check on the number of pills in the bottles. Urine tests were not considered, primarily because examination of urine for evidence of isoniazid could not be done without divulging to the staff that the populations of some villages were getting placebo.

Each week the representatives prepared brief statistical reports, the contents of which were condensed by the district nurse into one weekly report. The report was then telegraphically forwarded, together with supplementary remarks on the attitude of the population, and so forth, to the head of the sanatorium in Godthaab, who could thus constantly watch the course of the campaign.

The nurses were instructed to record on the individual cards any effect of the medication reported to them spontaneously. They were also allowed to ask neutral questions, according to the common rules of etiquette, about the general condition of health. They were not to ask leading questions, but persons refusing to continue to take the pills were, without provocation, to be asked the reason they would not continue.

Observations

Acceptability

The villages selected for the present study had a total population of 11,943 adult Greenlanders, 10,112 (85 per cent) of whom were included in the isoniazid trial. The 1,831 persons who were not included are accounted for in table 1. Most of them were not unwilling to participate but were excluded by the medical staff because of tuberculosis or other diseases. Only 723 persons (6 per cent) may be suspected of being unwilling to participate, as they were either absent from the roentgenographic examination, excluded without indication of reason or, in a few instances, frankly refused to participate. This 6 per cent is an overestimate, however, since many of those who did not turn up for the roentgenographic examination had valid excuses, such as being away temporarily for occupational reasons. Only 36 persons directly refused to participate. It therefore appears that the Greenland population was kindly disposed toward the project, and at least 95 per cent of those who were able to participate also were willing to do so.

The 10,112 adult Greenlanders who began taking the medication, and who thereby constitute the study population, are classified in table 2 according to sex, age, and whether assigned to the isoniazid or placebo group. The sex composition of the two groups is similar. The distribution by age is also practically alike in the two groups, although the proportion of women less than twenty-five years is somewhat larger in the isoniazid group than in the placebo group.

As indicated above, the population apparently was interested in participating. Another question was whether the participants could, and wanted to, remain with the project for the nine months for which it lasted in each village, or whether for

TABLE 1

Adult Population of 79 Villages in West Greenland

	Number	Per Cent
Total population	11,943	100.0
Total admitted to trial	10,112	84.7
Total excluded from trial	1,831	15.3
Active tuberculosis	1,040	8.7
Other medical reasons	68	0.6
No roentgenographic examination	562	4.7
Reason not given	125	1.3
Refused to participate	36	

TABLE 2

AGE AND SEX DISTRIBUTION OF 10,112 PERSONS ADMITTED TO THE TRIAL (STUDY POPULATION)

Age	Number				Per Cent			
	Isoniazid		Placebo		Isoniazid		Placebo	
	Men	Women	Men	Women	Men	Women	Men	Women
years								
14–19	520	616	522	487	21.9	22.2	22.2	18.7
20–24	346	436	371	360	14.5	15.7	15.8	13.8
25–34	597	613	563	646	25.0	22.1	24.0	24.8
35–44	407	432	389	468	17.1	15.6	16.6	17.9
45–54	268	354	267	342	11.2	12.8	11.4	13.1
55–	246	322	235	305	10.3	11.6	10.0	11.7
Total	2,384	2,773	2,347	2,608	100.0	100.0	100.0	100.0

some reason they would stop coming for the pills. If a person did not appear once or twice, he was not considered a defaulter; but if he failed to turn up several times, in spite of some reminders, he was then so classified. A total of 1,639 persons thus became classified as defaulters. They are distributed by reason for discontinuance and by study group in table 3. The most frequent reason for stopping the medication was absence from home; and two-thirds of the total number who discontinued had a valid excuse to stop, the reasons being death, disease, or travel. Only 490 persons discontinued because they did not come for the pills or because they refused to continue, i.e., 4.8 per cent of the persons who began taking the pills.

The total frequency of discontinuance owing to "absence" or "refusal" was greater in the isoniazid than in the placebo group, 6.2 per cent compared with 3.5 per cent.[3] On the other hand, discontinuance for various types of illness was less frequent in the isoniazid group than in the placebo group: 1.7 per cent and 3.2 per cent, respectively. A detailed analysis of morbidity in the two groups will be given in a later report.

As previously mentioned, the participants in the trial were in principle required to come each week for their ration of pills or, rather, a member of the household was to fetch the weekly ration. If the family had to show some initiative to get the pills, it was thought the pills would probably be taken. Through visits to the homes, examination of the pill boxes, and conversations with the families, a further attempt was made to ensure that the pills were being taken and to find out who, if any one, was not taking them. In this way some cases were found when there were doubts about whether the pills fetched were being consumed. There were more doubtful consumers in the isoniazid group than in the placebo group, 158 (3.1 per cent) and 56 (1.1 per cent), respectively.

[3] Analysis for the 13 largest villages, which included more than half of the total study population, showed a statistically significant village-to-village variation, within both groups, in the frequency of "absence or refusal," but the variation was about the same in the two groups.

TABLE 3

NUMBER AND PERCENTAGE OF PERSONS COMPLETING AND FAILING TO COMPLETE THE FULL COURSE OF MEDICATION

Study Population	Number		Per Cent	
	Isoniazid	Placebo	Isoniazid	Placebo
Total admitted to trial	5,157	4,955	100.0	100.0
Total completing medication	4,248	4,225	82.4	85.3
Total not completing medication	909	730	17.6	14.7
Died	18	18	0.3	0.4
Illness	89	161	1.7	3.2
Travel	446	331	8.6	6.7
Failure to fetch pills	171	85	3.3	1.7
Refused to continue	143	91	2.9	1.8
Side effects	42	44	0.8	0.9

Side Effects

In this trial, the participants were deliberately not questioned about special symptoms; only neutral inquiries were made about their general health, and those who at one time or another refused to continue the medication were asked if they had suffered any ill effects while taking

the pills. Complaints were registered on the individual record card for each person.

Symptoms were recorded for 54 of the 5,157 persons in the isoniazid group and for 62 of the 4,955 in the placebo group, a little more than 1 per cent in both groups. Neither the nurses nor the doctors found serious side effects in any of the persons: all the symptoms have been slight. Nevertheless, owing to certain symptoms, the doctors discontinued the pills in a total of 28 persons, 15 in the isoniazid group and 13 in the placebo group; and 58 persons (27 receiving isoniazid and 31 placebo) themselves refused to continue the treatment because of symptoms.

An analysis was made of the kind of symptoms of which people complained spontaneously, in view of the possibility of finding differences in the kind, frequency, and consequences of the symptoms between the isoniazid and placebo groups. In table 4 the complaints are summarized in ten categories. Six of the categories represent the subjective expression of the symptoms, whereas the categories representing dyspepsia, indisposition, paresthesia, and "various complaints" each comprise one or more complaints, as follows:

Dyspepsia: nausea, vomiting, stomach-ache.

Indisposition: indisposed, ill, unwell, cannot tolerate the pills, dare not take the pills, many sensations, discontent.

Paresthesia: prickly sensation of hands and feet, arm-ache, tongue ache, itching all over the body.

TABLE 4

COMPLAINTS REPORTED SPONTANEOUSLY DURING THE COURSE OF MEDICATION

Complaint	Isoniazid	Placebo	Total
Dyspepsia	25	26	51
Indisposition	10	13	23
Vertigo	12	9	21
Headache	10	8	18
Exanthema	10	6	16
Paresthesia	3	3	6
Heart symptoms	3	1	4
Pains (not localized)	2	1	3
Lessening of retentive powers	—	1	1
Various complaints	6	7	13
Total number of complaints	81	75	156
Total number of participants	5,157	4,955	10,112

Various complaints:

In isoniazid group: fever, nose-bleed, hemoptysis, fainting spells (had just had influenza), coughing.

In placebo group: hunger, thirst, catching colds, night perspiration, sore throat.

Apart from the "various complaints," which differed in the two groups, all of the symptoms in the isoniazid group also occurred in the placebo group, with little differences in frequency; this also applies to neurologic and psychic symptoms. In no case were serious side effects observed.

Only one person complained of the lessening of retentive powers and, curiously enough, this man was getting the placebo. He was a teacher, who, in addition to his teaching, had other intellectual work of great responsibility. In the first thirteen-week period of treatment he complained of being no longer able to remember normally.

In the isoniazid group, those who got 600 mg. per day did not complain more frequently than those who got only 400 mg. per day. Nor did the individual dose per kg. of body weight have any demonstrable effect: in the isoniazid group as well as in the placebo group, the distributions by weight were alike for those who had complaints and those who did not. During the interval of thirteen weeks between the first and second medication periods the symptoms disappeared with the same frequency in both groups.

These results might be taken as indicating that the side effects were absolutely independent of the substance isoniazid; but this is hardly true. A number of slight differences between the isoniazid and placebo groups suggest the possibility that in this general population, and with employment of intermittent dosage, isoniazid may have given specific side effects.

As is shown in table 5, complainers in the isoniazid group were more frequent among women than among men, but in the placebo group they were equally frequent among both sexes. For the isoniazid group, age seemed important: complainers were almost twice as frequent in persons more than thirty-five years as in persons less than thirty-five. In the placebo group, however, the frequency was the same in the older as in the younger groups. Of 54 complainers in the isoniazid group, 22 had more than one symptom; in the placebo group only 12 of the 62 complainers had. In the isoniazid group, 4 of the complainers

TABLE 5

SUMMARY OF SLIGHT DIFFERENCES BETWEEN THE ISONIAZID AND PLACEBO GROUPS

	Isoniazid			Placebo		
	Total Study Population	Complainers		Total Study Population	Complainers	
		Number	Per Cent		Number	Per Cent
Total	5,157	54	1.1	4,955	62	1.3
Sex						
Men	2,384	18	0.8	2,347	30	1.3
Women	2,773	36	1.3	2,608	32	1.2
Age						
14–34 years	3,128	25	0.8	2,949	36	1.2
35 or more	2,029	29	1.4	2,006	26	1.3
Failed to fetch pills or refused to continue medication	5,157	314	6.2	4,955	176	3.5
"Doubtful pill consumers"	5,157	158	3.1	4,955	56	1.1
	Total Number of Complainers	Number	Per Cent	Total Number of Complainers	Number	Per Cent
Persons with more than one symptom	54	22	40.7	62	12	19.4
Continued taking pills despite symptoms	54	4	7.4	62	12	19.4

continued taking the pills without interruption in spite of the symptoms, compared with 12 in the placebo group.

Furthermore, a higher proportion in the isoniazid than in the placebo group repeatedly failed to come for their pills, and more persons in the isoniazid group refused to continue taking the pills without giving a reason. "Doubtful pill consumers" were, as mentioned, also more frequent in the isoniazid group. These differences may be due to specific side effects of isoniazid, although none of the persons concerned complained of any ill effects while taking the pills.

The medication was discontinued by the local physicians in 28 per cent of the isoniazid group who complained of symptoms, and in 21 per cent of the placebo group with symptoms. In both groups psychic and allergic symptoms were the reason for discontinuation. Although dyspepsia was the most frequent complaint among all persons with symptoms, this symptom was reported in only two of the persons (one in each group) who were advised by a physician to discontinue the pills. No doubt the physicians' attitude toward the causality of the symptoms was decisive in discontinuing the medication, since they were not discouraged from assuming that all persons in the trial were receiving isoniazid in therapeutic doses, although they knew that the dose of isoniazid varied. The physicians feared the well-known neurologic and psychic side effects of large doses of isoniazid.

DISCUSSION

The question whether large-scale chemoprophylaxis will be a valuable, doubtful, or perhaps even dangerous public health procedure in combating tuberculosis ought to be solved within the next few years, as results can be obtained speedily in populations with a relatively high rate of tuberculosis morbidity. A major problem, however, in conducting investigations in such populations is that the conditions for identification and follow-up examination are usually poor. Greenland appears to be an exception. In spite of a good medical service, the Greenland population still has a very high rate of tuberculosis morbidity. Natural conditions and the great distances between the villages cause certain difficulties, but for a long time the population has been well registered, and since 1955 the regular tuberculosis control program has been supplemented with yearly roentgenographic examinations of the entire population. The situation in Greenland thus appears to offer an unusual opportunity for studying both the

immediate and long-term value of isoniazid prophylaxis on a mass scale.

Evidently almost the entire population was willing to begin the prophylactic treatment; and only 6.2 per cent of the participants in the isoniazid group and 3.5 per cent in the placebo group discontinued treatment on account of reluctance or indifference. Undoubtedly these percentages were not larger because, first of all, the Greenlanders are a friendly people and extremely interested in the fight against tuberculosis. They know that the disease is widespread in their country. Moreover, the people have always had great confidence in the Danish doctors and were therefore willing to cooperate. Another factor that may have had certain psychologic advantages was that the pills were to be taken only two days a week and the duration of each treatment period was only three months. This treatment scheme was based on results of Palmer's experimental studies, which appeared to the present writers to justify a trial of intermittent treatment with relatively large doses. The trials that were started in Italy about the same time also employed an intermittent treatment scheme with relatively large doses (11).

Acceptability of prophylactic medication is a difficult and important question, and numerous writers have discussed the matter of how to determine whether or not the pills are taken regularly. According to Debré and his associates, the French have so far regarded the regular reception of the pills as a reasonable checking method (14). Stott has discussed the acceptability question in some detail in connection with the prophylactic trials among suspect persons in Nairobi (15); and Fox has also written a critical review of the matter based on his experience in India (9). In the United States Public Health Service Trials (16), each household is supplied with pills in sufficient quantity for long periods of treatment, and the consumption is checked by counting the pills remaining in the bottles when unannounced visits are made to the homes. The same system is used in the Nairobi trials; and Stott has reported that among about 100 participants visited in their homes not more than one third could possibly have followed the prescribed regimen. Among the remaining two thirds, some had far too many pills left; others too few.

As far as side effects are concerned, Tørning (17) and Olsen and Tørning (12) have reported that ambulatory treatment of some patients with intellectually demanding work may cause serious neurologic and psychic disturbances. Results of the study of side effects in the present trial have some obvious limitations. For instance, the time of occurrence of symptoms could only rarely be precisely indicated because the distance between villages made it quite impossible for the nurses to call on all village representatives each week. Moreover, language difficulties sometimes interfered with exact registration of subjective symptoms; complaints of objective symptoms like skin eruption and edema caused no problems, of course. The important feature of this trial, however, is the presence of a control group for comparison with the isoniazid group. Between these two groups there should be no differences in the shortcomings of the registration of symptoms. Moreover, subjective symptoms were only registered if they were spontaneous complaints: no direct inquiries were made for the purpose of eliciting complaints. While the control group might be criticized from the point of view that some persons may have an idiosyncracy to quinine sulfate, or that the very small dose of isoniazid in the placebo pills may have caused some allergic sensitivity, the frequency of that type of reaction is probably too low to be of much consequence.

Almost all of the symptoms in both groups were nonspecific, and the kind that many people, especially the elderly, have in daily life, and they seem to be related to different external causes. Although the frequency of symptoms differed little in the isoniazid and control groups, there are several indications that side effects actually may have been more frequent in the isoniazid group, and that they differed in some respects from those in the placebo group. However, no serious side effects occurred in either group; and, although all of the persons who refused medication may possibly have had certain subjective symptoms which they did not want to mention to the representative or the nurse, the total frequency of side effects was so low that chemoprophylaxis with the doses used here apparently can be carried through without risk in a general population.

A final conclusion on the question of whether the relatively small weekly doses of isoniazid, given for two periods of three months each, as in the present trial, can be used as chemoprophy-

laxis in a general adult population like that of Greenland will have to await an evaluation of the potential harm caused by development of isoniazid-resistant bacilli in the population in comparison with the potential benefit of prophylaxis in reducing tuberculosis morbidity.

Summary

In July, 1956, a control study of prophylactic isoniazid was started in the general adult population of 79 villages in West Greenland. By random allocation of the villages into two groups, about equal numbers of persons started taking either isoniazid or placebo, 5,000 in each group. Both types of pills were identical in appearance and taste; they were taken two days a week for two periods of thirteen weeks each, separated by an interval of thirteen weeks. The daily dose of isoniazid was 400 mg. for the majority and 600 mg. for a small subgroup.

Although the weekly acquisition of pills was considered an indication that the pills were being taken, unscheduled visits were also made to the homes to check the supply on hand. Despite uncertainties, pill consumption seemed to be carried out satisfactorily by 82 per cent of the isoniazid group and 85 per cent of the placebo group. The most frequent reason for discontinuing the pills in both groups was travel, usually occupational.

Spontaneous complaints of symptoms were made by about 1 per cent of each group. The symptoms were similar in both groups. Although no serious side effects occurred in either group, some indications were found that isoniazid may have caused specific subjective side effects in some persons.

Sumario

Quimioprofilaxis Colectiva de la Tuberculosis: La Aceptabilidad y Efectors Laterales Adversos de la Isoniacida en un Estudio Fiscalizado en Groenlandia

En julio de 1956, se inició un estudio fiscalizado de la isoniacida como profiláctico en la población adulta general de 79 aldeas de la porción occidental de Groenlandia. Por distribución al azar de las aldeas en dos grupos, aproximadamente números iguales de personas comenzaron a tomar isoniacida o un placebo, 5,000 en cada grupo. Ambas clases de píldoras eran idénticas en aspecto y sabor; se tomaron dos días por semana durante dos períodos de 13 semanas cada uno, separados por un intervalo de 13 semanas. La dosis diaria de isoniacida fué de 400 mg. para la mayoría y de 600 mg. para un pequeño sub-grupo.

Aunque se consideró la adquisición semanal de píldoras como indicación de que se tomaban las mismas, también se hicieron visitas inesperadas a los hogares para averiguar qué repuestos había a mano. A pesar de las incertidumbres, el consumo de píldoras pareció ser satisfactorio en lo tocante a 82 por ciento del grupo de la isoniacida y 85 por ciento del grupo del placebo. En ambos grupos, la causa más frecuente del abandono de las píldoras procedió de los viajes, por lo general relacionados con la ocupación.

Hubo quejas espontáneas de síntomas aproximadamente por 1 por ciento de cada grupo. Los síntomas fueron semejantes en ambos grupos. Si bien no hubo ni en uno ni otro grupo graves efectos colaterales, se observaron algunas indicaciones de que la isoniacida pudo haber producido efectos colaterales subjetivos específicos en algunas personas.

Resume

Chimioprophylaxie massive contre la tuberculose: tolérance et effets défavorable de l'isoniazide dans une étude de contrôle au Gröenland

En Juillet 1956, une étude de contrôle de prophylaxie par l'isoniazide fut commencée parmi toute la population adulte de 79 villages de l'ouest du Gröenland. Après une séparation arbitraire des villages en deux groupes, un nombre à peu près égal de personnes commençèrent à prendre soit de l'isoniazide, soit des "placebo," 5000 dans chaque groupe. Les deux sortes de pilules étaient identiques d'apparence et de goût; elles furent prises deux jours par semaine pendant deux périodes de 13 semaines chacune, séparées par un intervalle de 13 semaines. La dose journalière d'isoniaside fut de 400 mg pour la majorité des sujets et 600 mg pour un petit sous-groupe.

Bien que l'on estima que l'acquisition hebdomadaire de pilules indiquait que les pilules étaient absorbées, des visites à l'improviste furent aussi effectuées à domicile pour vérifier ce qui restait de la provision de pilules. Malgré certains doutes la consommation des pilules parut être satisfaisante chez 82% du groupe à l'isoniazide et chez 85% du groupe aux "placebo." La raison la plus fréquente d'arrêt des pilules fut, dans les deux groupes, des voyages, génèralement des voyages d'affaires.

A peu près 1% de chaque groupe se plaignit de symptômes. Les symptômes furent semblables dans les deux groupes. Bien que nulle réaction

sérieuse ne se présenta dans aucun groupe, on trouva quelques faits indiquant que l'isoniazide a pu causé des réactions subjectives spécifiques chez quelques sujets.

Acknowledgments

This project could not have succeeded without the excellent team work of the physicians, nurses, and secretaries, to all of whom the writers are very grateful.

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THE PRODUCTION OF LYSINE FROM DIAMINOPIMELIC ACID BY CELL-FREE EXTRACTS OF MYCOBACTERIUM TUBERCULOSIS[1, 2]

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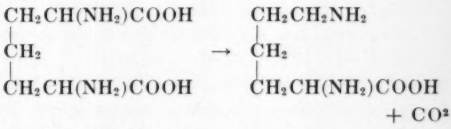
Introduction

In an investigation of the amino acids of *Corynebacterium diphtheriae* by paper chromatography techniques, Work (1, 2) detected in acid hydrolysates of the cells a new amino acid, subsequently isolated and identified as α-ϵ-diaminopimelic acid (DAP). This amino acid has since been found in nearly all of the bacteria examined except for the gram-positive cocci. In many it constitutes a vital part of the amino acid composition of the cell wall (3). A specific decarboxylase for diaminopimelic acid, which breaks it down to lysine and carbon dioxide, has been demonstrated in a number of coli-aerogenes organisms (4). It has been postulated that in those strains possessing DAP decarboxylase, DAP is an intermediate in the synthesis of lysine (5).



Diaminopimelic acid Lysine

Although diaminopimelic acid has been demonstrated in hydrolysates from antigenic lipopolysaccharides of *Mycobacterium tuberculosis* (6), from cell walls of these organisms (7), and has been isolated from whole tubercle bacilli (8), there has been no report of a metabolic role for this amino acid in tubercle bacilli. In this paper the demonstration of the enzyme diaminopimelic acid decarboxylase in cell-free extracts of *M. tuberculosis* and the optimal conditions for enzyme activity are described.

[1] From the Department of Microbiology, Duke University Medical Center, Durham, North Carolina.

[2] This study was aided by a grant from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association.

Materials and Methods

The organisms employed in this study were the R1Rv, H37Rv, and H37Ra strains, maintained by biweekly transfer of surface growth on Vorwald's modification of the Proskauer-Beck liquid synthetic medium, pH 7.0.

Cells used as the source of enzyme were also grown on this medium in 480-ml. bottles containing 0.5 per cent asparagin, 0.5 per cent KH_2PO_4, 0.25 per cent Mg^{++} citrate, 0.06 per cent anhydrous $MgSO_4$, and 2.5 per cent glycerol. The inoculum was from fourteen-day stock cultures, floated on the surface of the medium and incubated at 37°C. in a horizontal position for three weeks. The cells were harvested by decanting the culture medium; were washed twice in sterile cold distilled water and centrifuged for one hour in a refrigerated centrifuge. The cells were resuspended in a small amount of 0.1 M potassium phosphate buffer, pH 7.2, and ground in a Ten Broeck tissue grinder in order to break up the large clumps. Additional buffer was added and the cells were again centrifuged for one hour before suspension in a volume of buffer to contain approximately 65 mg. (dry weight) per milliliter.

Extracts of the cells were obtained by exposure for one hour in a Raytheon 9 KC sonic oscillator in which the water that bathed the vibrating shaft was chilled by passage through a copper coil submerged in crushed ice. The straw-colored supernatant, obtained by centrifugation at 16,000 × G for one hour, contained the active enzyme and was used immediately. Because of the long reaction time employed, sterile conditions were maintained throughout all of the experiments.

Enzymatic methods: Unless otherwise stated, for the detection of enzymatic activity, the reaction mixture contained 12.5 mM of synthetic diaminopimelic acid, 10 γ of pyridoxal phosphate, 0.5 ml. of cell-free extract containing approximately 1 mg. of nitrogen in a total reaction volume of 2.6 ml. All solutions were made up in 0.1 M potassium phosphate buffer, pH 7.2, using deionized water. Suitable controls in which substrate or enzyme was omitted, or in which the reaction was stopped at 0-time, were included in all experiments.

The reactions were carried out in 25-ml. Erlenmeyer flasks in a thermostatically controlled water-bath shaker at 37°C. for eighteen hours. The reaction was stopped by heating at 120°C. for five minutes, followed by centrifugation to remove the precipitated protein. The clear super-

natant was examined for enzymic reaction products.

The presence of lysine was detected by one-dimensional paper chromatography, using Whatman No. 3 mm. filter paper and the descending technique. Phenol-water (100:20) and butanol-acetic acid-water (250:60:250) were used as solvents. When quantitative data were desired, the chromatogram was developed for eighteen hours in butanol-acetic-water, dried, and returned to fresh butanol-acetic-water for an additional eighteen hours. The intensity of ninhydrin reacting spots was determined by use of the Spinco analytron. Final identification of lysine and quantitative information were obtained by microbiologic assay using *Leuconostoc mesenteroides* #8042 as the test organism.

Observations

In cell-free extracts of the three strains of tubercle bacilli studied, an enzyme was detected which converted diaminopimelic acid to lysine. Coincident with the disappearance of diaminopimelic acid in reaction mixtures, a second amino acid, lysine, appeared. The identification of lysine was based on the finding by paper chromatography of a ninhydrin-reacting spot which moved at the same rate as a known sample of lysine, and on the finding that the lysine requirements of the organism *L. mesenteroides* were fulfilled by a substance present in the DAP reaction mixtures but not in the controls.

Characterization of the enzyme responsible for the conversion of diaminopimelic acid to lysine has been made by a study of the optimal condition for activity. Diaminopimelic acid decarboxylase activity of the R1Rv strain was not increased by the addition of 100 γ of pyridoxine or pyridoxal or of 20 mg. of DAP per 100 ml. of culture medium. Consequently, in all of the succeeding experiments, organisms were grown on the basic synthetic medium without supplements.

In order to determine the effect of time on the enzyme reaction, an experiment was conducted in the presence and absence of pyridoxal phosphate (100 γ per reaction flask) in which the reaction of duplicate reaction mixtures was stopped at various time intervals from thirty minutes to twenty-two hours. It may be seen in figure 1 that from the beginning the rate of diaminopimelic acid decarboxylation is greater in the presence of extraneous pyridoxal phosphate and that this difference becomes more pronounced with the passage of time. There is

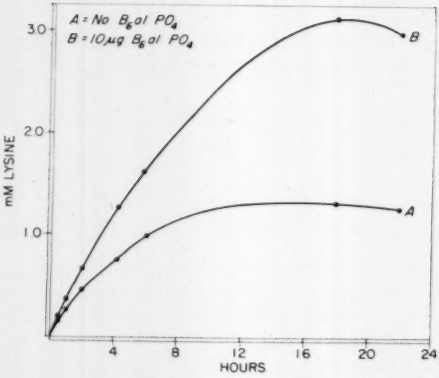


FIG. 1. The effect of reaction time on diaminopimelic acid decarboxylase activity.

also an earlier leveling off of lysine production in the absence of pyridoxal phosphate. On the basis of these findings, in which eighteen hours were required for maximal conversion of DAP to lysine in the presence of pyridoxal phosphate, this time period was employed in all future experiments.

Another factor studied was the effect of the length of exposure in the sonic oscillator to enzyme activity. Tubercle bacilli are extremely resistant to disintegration when methods effective for other organisms are used. They can, however, be ruptured by prolonged exposure in a sonic oscillator. Since many enzymes are inactivated by relatively short exposure to sound waves, it was necessary to determine the time period required for breaking up a considerable number of organisms without loss of enzyme activity. Samples were removed from the oscillator following vibration for various periods. Before removal of intact cells and particulate matter, Ziehl-Neelsen–stained smears were made of each sample. The decarboxylase activity of these preparations is presented in table 1, showing an increase in lysine production as the vibration time was increased. Stained smears of samples *A* and *B* revealed that the majority (about 90 per cent) of the cells were not disintegrated (as evidenced by their continuing to stain acid fast), and that only a small number of nonacid-fast forms were present. In the *C* preparation, vibrated for sixty minutes, although a large amount of nonacid-fast fragments were seen, about 50 per cent of the cells were still acid fast. Approximately 25 per cent were acid fast in

TABLE 1

THE EFFECT OF VIBRATION TIME ON ENZYME ACTIVITY

Sample	Vibration Time	Lysine
	minutes	γ
A	15	528.6
B	30	538.9
C	60	618.2
D	90	665.7
E	120	850.2

the *D* sample, scattered throughout a large amount of amorphous blue fragments, while in the *E* preparation exposed for two hours only about 5 per cent remained acid fast, and these were present not in large massive clumps but were evenly distributed throughout the blue field. Although an increase in diaminopimelic acid decarboxylase activity was observed as the vibration time was increased, the sample that was vibrated only fifteen minutes possessed 62 per cent of the activity of the preparation vibrated for two hours. Considerable loss of enzyme activity accompanied prolonged vibration, but this was partially compensated for by a release of active enzyme as new cells were disrupted. Loss of activity during a one-hour vibration period could be reduced approximately 25 per cent by the addition of 1×10^{-4} M BAL (2:3 dimercaptopropanol) to the suspending buffer.

The effect of pH on activity of the enzyme is shown in figure 2. There is a sharp rise in enzyme activity as the pH is increased from 6.2 to 7.2, when the maximal activity occurs. This is the same optimal pH obtained by Dewey and coworkers (9) for *E. coli* decarboxylase in contrast to the 6.8 optimum for *A. aerogenes*.

Another factor studied was the effect on enzyme activity of age of the cells at the time of harvesting. Cells were harvested after a growth period of two, three, four, five, six, and eight weeks, cell-free extracts were prepared and tested for diaminopimelic acid decarboxylase activity in the presence and absence of extraneous pyridoxal phosphate. The findings are presented in table 2.

Mycobacterium tuberculosis is a very slow-growing organism in which maximal growth on synthetic medium is usually not attained until about the sixth week. When these organisms were tested either in the absence or presence of extraneous pyridoxal, there was a progressive increase in the amount of lysine produced from the second through the sixth weeks as a result of the greater number of cells from which the enzyme was extracted. The availability of more apoenzyme through this period resulted in the progressive increase of stimulation of decarboxylase activity by pyridoxal phosphate. Extracts from eight-week cells were very low in activity in the absence of added pyridoxal, although this activity was raised almost to the six-week level by the addition of 10 γ of pyridoxal phosphate. When calculations were made on the basis of γ of lysine synthesized per mg. of nitrogen in the cellular extract, a similar relationship was obtained in the absence of the extraneous pyridoxal; but with added pyridoxal the decline in eight-week cells was not observed. This indicated that, although cell autolysis was occurring at a rate greater than cell synthesis, activity of the apoenzyme when additional coenzyme was supplied was still very high.

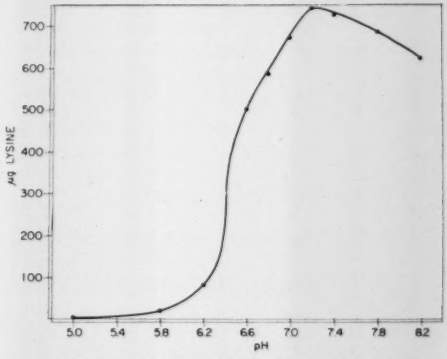


FIG. 2. The effect of pH on diaminopimelic acid decarboxylase activity.

TABLE 2

THE EFFECT OF AGE ON DIAMINOPIMELIC ACID DECARBOXYLASE ACTIVITY IN TUBERCLE BACILLI

Age of Cells	γ Lysine per Ml.		Per Cent Stimulation
	No B_6al PO_4	10 γ B_6al PO_4	
weeks			
2	56.4	82.5	46.3
3	117.5	193.1	64.3
4	167.3	302.8	81.0
5	187.9	400.4	113.1
6	209.8	458.1	118.3
8	79.7	414.2	419.7

TABLE 3

THE EFFECT OF ENZYME CONCENTRATION ON DIAMINOPIMELIC ACID (DAP) DECARBOXYLASE ACTIVITY

Enzyme Concentration	mM Lysine per Ml.		Per Cent Stimulation by B_6al PO_4
	No B_6al PO_4	B_6al PO_4 Added	
mg. N per ml.			
0.045	0.099	0.210	113.2
0.225	0.529	0.876	65.6
0.450	1.046	1.519	45.3
0.900	2.099	2.792	33.0

12.5 mM DAP; 10 γ B_6al PO_4; total reaction volume = 2.6 ml.

TABLE 4

THE EFFECT OF SUBSTRATE CONCENTRATION ON DIAMINOPIMELIC ACID (DAP) DECARBOXYLASE ACTIVITY

Concentration of DAP	Concentration of Lysine	Per Cent DAP Utilization
mM	*mM*	
1.25	0.49	39.2
1.25 + 10 γ B_6al PO_4	0.76	60.8
6.25	1.85	29.6
6.25 + 10 γ B_6al PO_4	2.80	44.8
12.5	2.81	22.5
12.5 + 10 γ B_6al PO_4	3.95	31.6
25.0	4.48	17.9
25.0 + 10 γ B_6al PO_4	5.08	20.3

0.5 ml (588.5 γ N) cell-free extract; total reaction volume = 2.6 ml.

TABLE 5

THE EFFECT OF PYRIDOXAL PHOSPHATE ON DIAMINOPIMELIC ACID (DAP) DECARBOXYLASE ACTIVITY

Moles of B_6al PO_4	mM of Lysine	Per Cent Stimulation by B_6al PO_4
0	2.89	
2×10^{-6}	3.56	23.2
5×10^{-6}	3.95	36.7
1×10^{-5}	4.63	60.2
2×10^{-5}	5.30	83.4
5×10^{-5}	8.16	182.4
1×10^{-4}	8.79	204.2
2×10^{-4}	9.54	230.1

12.5 mM DAP; 0.5 ml. (0.85 mg. N) cell-free extract; total reaction volume = 2.6 ml.

The effect of increasing the concentrations of enzyme, substrate, and pyridoxal phosphate on decarboxylase activity is shown in tables 3, 4, and 5. An increase in the concentration of each of these factors within the range tested resulted in an increased production of lysine. Maximal stimulation by pyridoxal phosphate was observed at the lower enzyme and substrate levels. Increasing the concentration of pyridoxal brought about a marked increase in enzyme activity. Maximal utilization of substrate was obtained at the 1.25 mM concentration level. Considerable unchanged diaminopimelic acid was detectable by paper chromatography in the reaction mixture, especially at the higher levels.

The effect of several compounds related either to diaminopimelic acid or to pyridoxal phosphate was tested on enzyme activity. These included pyridine-2:6-dicarboxylic acid (dipicolinic acid), adipic acid diamide, pimelic acid, isoniazid, and desoxypyridoxine. The enzyme was without activity on these compounds when tested with extracts from H37Ra and R1Rv organisms in the absence of diaminopimelic acid.

In the presence of diaminopimelic acid, adipic acid diamide, pimelic acid, and desoxypridoxine exerted neither an inhibitory nor stimulatory effect on decarboxylase activity. Dipicolinic acid in concentrations of 5 to 25 mM resulted in an approximately 50 per cent increase in decarboxylase activity (table 6). Stimulation was observed both in the presence and absence of pyridoxal phosphate.

Isoniazid exerted a marked inhibitory effect

TABLE 6

THE EFFECT OF DIPICOLINIC ACID ON DIAMINOPIMELIC ACID (DAP) DECARBOXYLASE ACTIVITY

Concentration of Dipicolinic Acid	No B_6al PO_4		B_6al PO_4 added	
	Lysine	Per Cent Stimulation	Lysine	Per Cent Stimulation
mM	*γ/ml.*		*γ/ml.*	
0	89.0		348.0	
0.5	103.2	15.9	375.8	8.0
1.0	108.6	22.0	416.9	19.8
5.0	134.2	50.7	494.2	42.0
12.5	134.9	51.5	506.9	45.7
25.0	135.3	51.9	504.9	45.1

12.5 mM DAP; 5×10^{-5} M B_6al PO_4; Reaction volume = 2.6 ml.

on diaminopimelic acid decarboxylase activity when tested in concentrations of 1 to 100 γ per reaction mixture. This effect was most pronounced when the drug was preincubated with the enzyme-containing extract for one hour before the addition of substrate and pyridoxal phosphate. Preincubation with the coenzyme, however, markedly decreased the degree of inhibition by isoniazid and, when sufficiently high concentrations were employed, completely protected against the drug. As higher levels of the drug were used, it was necessary to employ higher pyridoxal phosphate concentrations (table 7).

TABLE 7

THE EFFECT OF PYRIDOXAL PHOSPHATE ON INHIBITION OF DIAMINOPIMELIC ACID (DAP) DECARBOXYLASE BY ISONIAZID

Isoniazid Concentration	$B_6al\ PO_4$ Concentration	A		B	
		Lysine	Inhibition	Lysine	Inhibition
γ	γ	γ	*per cent*	γ	*per cent*
0	0	128.1		132.5	
	1	137.2		135.8	
	10	180.8		178.9	
	100	320.9		328.8	
1	0	123.1	3.9	115.3	13.0
	1	134.5	2.0	119.6	11.9
	10	180.1	0	156.0	12.8
	100	321.5	0	327.6	0
10	0	95.5	25.4	71.7	45.9
	1	99.8	27.3	65.7	51.6
	10	137.0	24.2	85.4	52.3
	100	379.1	0	234.9	28.6
100	0	19.5	84.8	3.6	97.3
	1	19.0	86.2	1.4	99.0
	10	28.3	84.3	1.3	99.3
	100	317.4	1.1	41.8	87.3

A = 1-hour preincubation with $B_6al\ PO_4$; B = 1-hour preincubation with isoniazid.

TABLE 8

DIAMINOPIMELIC ACID (DAP) DECARBOXYLASE ACTIVITY OF VIRULENT AND AVIRULENT STRAINS OF TUBERCLE BACILLI

Strain	γ Lysine per Ml.	
	No $B_6al\ PO_4$	$B_6al\ PO_4$ added
H37Rv (virulent)	182.3	470.8
H37Ra (avirulent)	149.1	473.7
R1Rv (avirulent)	162.8	459.6

12.5 mM DAP; 5×10^{-5} M $B_6al\ PO_4$; reaction volume = 2.6 ml.

In a comparison of the diaminopimelic acid decarboxylase activity of three representative strains of virulent and avirulent tubercle bacilli, no significant differences were detected between the diaminopimelic acid decarboxylase activities of the three strains tested (table 8). Cells used as the source of enzyme were of comparable age, grown under uniform conditions, and had a comparable nitrogen content.

When protected from the light and placed in a closed carton stored at −20°C., cell-free extracts retained their diaminopimelic acid decarboxylase activity. Extracts held in this manner for periods up to sixteen weeks were equally as competent as the freshly prepared extract. Partial inactivation occurred when the extract was dialyzed against buffer for twenty-four hours, but this loss of activity could be reduced to approximately 11 per cent when the dialyzed extract was tested in systems containing pyridoxal phosphate, or to 25 per cent when tested in its absence. Dialysis failed to result in dissociation of the apoenzyme-coenzyme complex. Attempts to purify the enzyme by acetone precipitation of dialyzed extracts have resulted in preparations of low decarboxylase activity.

DISCUSSION

Tubercle bacilli have long been known to be capable of growth on a synthetic medium, synthesizing their entire body substance from a few simple compounds. All of the commonly occurring amino acids, as well as diaminopimelic acid, have been identified in a number of different analyses of the bacillary bodies. There have been few studies on the pathways employed in the synthesis of these amino acids, and in none is there an indication of the precursor or precursors in the formation of lysine. Transaminase studies in this laboratory on tubercle bacilli, using intact cells and cell-free extracts, have indicated that lysine is not an important amino donor in the system (10). Lysine is one of the hexone bases, however, found in relatively high concentrations in cells and filtrates from all strains of tubercle bacilli investigated, and in a recent study was observed to be present in smaller amounts in filtrates of an isoniazid-resistant strain than in its isoniazid-susceptible parent (11).

The finding of an enzyme in cell-free extracts of tubercle bacilli which converts diaminopimelic acid to lysine and carbon dioxide suggests that this pathway is important in the lysine metabolism of this organism. Diaminopimelic acid decarboxylase differs from most of the other known bacterial amino acid decarboxylases in a number of respects. Its activity is not affected by the addition of DAP to the culture medium; it is produced by young as well as by aging cells; and its optimal pH is near neutrality, at which pH growth of the organism itself is best. The end product of the decarboxylation, lysine, is of great metabolic importance, whereas the basic amines produced by the other amino acid decarboxylases are formed as an adaptive defense against an acid environment and have no major metabolic significance. It would appear that, in tubercle bacilli as well as in *E. coli* as reported by Dewey and co-workers (9), diaminopimelic acid decarboxylase is a constitutive enzyme, serving an important role in lysine synthesis.

The decarboxylase from tubercle bacilli resembles that of *A. aerogenes* in that the age of the cells at the time of harvesting is not a critical factor and good activity is observed in cells two to eight weeks old. Its optimal pH of 7.2 is similar to that observed for *E. coli* (9).

Although decarboxylation by cell-free extracts did not require added pyridoxal phosphate, its addition increased the activity of all preparations tested. The amount of stimulation varied with the different extracts, but its effect is especially pronounced on extracts prepared from aging cells eight weeks old. Pyridoxal also restored to full activity cell-free preparations stored in an open rack at −20°C. for four weeks. Although dissociation of pyridoxal phosphate from its apoenzyme by dialysis was not effected, the marked stimulation achieved by pyridoxal phosphate is strongly indicative of a coenzyme function.

Of the analogues of pyridoxal and diaminopimelic acid tested, only isoniazid exerted an inhibitory effect. Fifty per cent inhibition was obtained when 10 γ of the drug were preincubated for one hour with the extract, but preliminary incubation with the coenzyme or the simultaneous addition of isoniazid and pyridoxal phosphate reduced the degree of inhibition to 25 per cent. As the concentration of the drug was increased, it was necessary to increase the concentration of pyridoxal phosphate required to neutralize the inhibition.

It has been shown previously that pyridoxal is also capable of reversing the antituberculous activity of isoniazid (12). This observation, together with the finding that lysine synthesis is inhibited by culture of the organism in the presence of subinhibitory concentrations of isoniazid and that lysine production by an isoniazid-resistant strain is significantly lower than that by its isoniazid-susceptible parent (11), strongly suggests that the inhibition of at least one pyridoxal-requiring enzyme, diaminopimelic acid decarboxylase, contributes to the antituberculous activity of the drug. Further studies are necessary to delineate the exact mechanism by which isoniazid effects inhibition of this reaction.

Summary

Cell-free extracts prepared from the R1Rv, H37Rv, and H37Ra strains of *M. tuberculosis* have been shown to possess an enzyme capable of decarboxylating diaminopimelic acid. Products of the reaction are lysine and carbon dioxide. No differences could be detected in the activity between extracts of the virulent and avirulent strains.

In a study of the influence of various factors on enzyme activity, a pH of 7.2 was found to be optimal. Although pyridoxal phosphate was not essential for enzyme activity in the system studied, its addition markedly stimulated lysine formation.

Isoniazid inhibited diaminopimelic acid-decarboxylase activity, but this inhibition could be overcome by the addition of pyridoxal phosphate.

Sumario

La Producción de Lisina del Acido Diaminopimélico por Extractos Citoprivos del Mycobacterium Tuberculosis

Según se ha demostrado, los extractos exentos de células de las cepas R1Rv, H37Rv y H37Ra del *M. tuberculosis* poseen una enzima capaz de descarbolizar ácido diaminopimélico. Los productos de la reacción son lisina y bióxido de carbono. No pudo distinguirse ninguna diferencia de la actividad entre los extractos de las cepas virulentas y las avirulentas.

En un estudio del influjo de varios factores sobre la actividad enzimática, se descubrió que un pH de 7.2 resultaba óptimo. Aunque el fosfato de piridoxal no fué indispensable para obtener

actividad enzimática, su adición excitó notablemente la lisinogenia.

La isoniacida inhibió la actividad del ácido diaminopimélico-des-carboxilasa, pero podía superarse esa inhibición con la adición de fosfato de piridoxal.

Resume

La production de lysine à partir de l'acide diaminopimelique produit par des extraits de "cellules libres" de Mycobactérium tuberculosis

On a montré que des extraits de "cellules libres" de mycobactéries tuberculosis, en particulier des souches RIRv, H37Rv, et H37Ra, possèdent un enzyme capable de décarboxyler l'acide diaminopimelique. Les produits de la réaction sont la lysine et du dioxyde de carbone. On n'a pu trouvé aucune différence d'activité entre les extraits des souches virulentes et non virulentes.

Dans une étude sur l'influence de différents facteurs sur l'activité enzymatique, on a trouvé que le pH optima était 7.2. Bien que le phosphate de pyridoxal ne soit pas essentiel à l'activité enzymatique du système étudié, son addition stimule notablement la formation de lysine.

L'isoniazide inhibe l'activité de la décarboxylase de l'acide diaminopimelique, mais cette inhibition put être surmontée par addition de phosphate de pyridoxal.

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IDENTIFICATION OF "POSITIVES" IN ROENTGENOGRAPHIC READINGS[1]

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INTRODUCTION

This report of an investigation into the reliability of roentgenographic readings is one of a series of similar investigations that followed the publication in 1947 (1) of a now classic study initiated by Yerushalmy. That study had as its object the comparison of several types of roentgenograms (14- by 17-inch film, 14- by 17-inch paper roentgenogram, 4- by 10-inch stereophotofluorogram, 35-mm. photofluorogram) for use in mass surveys conducted to discover persons with pulmonary tuberculosis. It revealed variation in the readings with each type of roentgenogram. Even with the 14- by 17-inch film, generally considered the most accurate, the variation appeared amazingly large to those not already acquainted with the wide statistical variability of clinical tests. Each of five expert roentgenologists who participated in the experiment showed wide differences in reading the various types of roentgenograms for the same patient; the five roentgenologists differed among themselves in reading the same roentgenogram; and each roentgenologist even differed significantly with himself in reading the same roentgenogram at different times. Within the range of variation experienced with each roentgenographic method, no difference could be discerned between them with regard to effectiveness in mass tuberculosis survey work. Interest and concern were aroused.

There followed, in a series of papers by a group of distinguished radiologists and specialists in diseases of the thorax, what is doubtless the most painstaking and self-critically objective examination on record by physicians of the imprecision of their own professional technique. Investigations were made of the reliability of particular types of roentgenograms for many varieties of problems, such as the discovery of cases of pneumoconiosis and the likelihood of missing progressive cases, the reliability of serial standard films for the recognition of progression or remission of pulmonary tuberculosis, the classification and prognostic significance of different types of shadows, and the use of miniature photofluorograms for routine physical examination. Depending on the objective, these studies portrayed different aspects of reliability, and the appended bibliography may be consulted for the details. All are in agreement in showing that wide differences are to be expected in the reading of roentgenograms, even by the most expert roentgenologists, with any type of roentgenogram and whatever the diagnostic problem. However, difference of opinion has been expressed with regard to the seriousness of the existing variation for clinical practice (2).

The present investigation was undertaken to estimate the reliability of reading "chest films" (postero-anterior roentgenograms of the thorax made on 14- by 17-inch film), especially of patients referred for roentgenographic study in the course of medical examination in a large clinic. Two types of readings were considered: that of the single roentgenogram and that of stereoscopic roentgenograms.

The rationale and method of the study may be set forth as follows:

> The objective was limited to the designation of a roentgenogram as "positive" or "negative." "Positive" means the presence of a shadow indicating a pathologic condition of the lung; "negative" means the absence of such a shadow. The conception is that there are roentgenograms which, if studied with sufficient care by competent roentgenologists, will be found to be "positive." Such roentgenograms may be read erroneously by a particular roentgenologist, but, if the pathologic shadow is indicated to him, he will agree on the positivity.

Of course, in reality, there are roentgenograms that do not conform to this conception. No matter how carefully they are studied, by an individual roentgenologist or by several in consultation, complete agreement will not result—some will definitely consider the roentgenogram

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positive, some negative. Such roentgenograms are considered "doubtful" and are not included in this study. The object of the present investigation is to ascertain the reliability of the reading of such "positives" and "negatives" when this is done in the usual manner of viewing a large number of roentgenograms successively as a diagnostic aid in clinical practice.[2]

Material and Methods

The plan was to designate about 100 positive and 400 negative roentgenograms of the thorax to comprise the series to be reviewed.[3] Two examiners, one a roentgenologist and the other a specialist in diseases of the thorax, examined together a large number of roentgenograms of adults and selected the series for the study. For the positive group, only roentgenograms with definitely abnormal shadows indicating pulmonary disease were accepted. All shadows were minimal in size, varying from 1 to 2.5 cm. in diameter. Some were sharply circumscribed and others were not. Lesions in all parts of the lungs were included, but each positive roentgenogram contained only one abnormal shadow. For the negative group of roentgenograms, all those with suspicious minimal shadows or artifacts were excluded. Roentgenograms with a calcified Ghon tubercle or a calcified lymph node were accepted and considered negative. No attention was paid to obvious anomalies of the ribs, abnormal cardiac shadows, or abnormal shadows below the diaphragm or in the neck.

The roentgenograms were arranged in random order by use of a table of random numbers, the original case history number having been concealed by an opaque label. Nine readers participated in the experiment, five of whom were roentgenologists and four, specialists in diseases of the thorax.[4] They did not know the number of positive films included. Each reader reviewed the series of single roentgenograms first, reading them successively in the way they are read when they are viewed for the clinical assessment of patients. The diagnosis of positive or negative was dictated to a clerical assistant who recorded the data for later transfer to punch cards. After completion of the reading of the single roentgenograms, each was matched with its corresponding stereoscopic roentgenogram and the series of 500 sets were then arranged in a new random order. The experiment was repeated as with the single roentgenogram, but this time each pair was viewed stereoscopically. A period of several months elapsed between the viewing of the roentgenograms singly and stereoscopically. The results are reported separately for the positive and for the negative roentgenograms.[5] If, in reading a positive roentgenogram, the reader designated it as negative, this is referred to as a "false negative." Similarly, a negative roentgenogram diagnosed as positive is designated as a "false positive."

[2] The problem here thus differed basically from the problem dealt with mathematically by Neyman, in which it was undertaken to estimate, from the results of the readings alone, the proportions of the population with varying degrees of disease, as well as the percentages of those correctly diagnosed. Similarly, it differed in its objective from investigations into the reliability of miniature roentgenograms employed in mass survey work. The numerical results reported here apply only to the kind of cases and the particular methods that were used in the present investigation.

[3] In some other investigations, the ratio of positive to total roentgenograms is much smaller than this, being in the order of 1 to 2 per cent instead of 20 per cent, as in the present case. The reason is that, in the investigations referred to, the positive roentgenograms are taken in approximately the ratio in which they are found; and the groups examined are, for instance, college students undergoing routine physical checkup. It is generally considered desirable to have the ratio of positive roentgenograms about that of the series examined, but this usually results in a small number of positive roentgenograms, even if the total series is very large. Since the most important element of the investigation is to assess the ability to recognize positive cases, the presence in the study of only a small number of such cases may be considered a serious defect. It is for this reason that it was decided to include about 100 positive cases. It is believed that the intermixing of these at random among 400 negatives simulates sufficiently closely the conditions of practice to assure a fairly representative record of the percentage of false positive readings.

[4] The two examiners who chose the positive and negative roentgenograms are included among the nine, namely readers *C* and *D* in tables 1 through 5. It naturally suggested itself that these should be omitted from the report. However, on careful consideration, it was decided that a more representative set of results would be obtained if these were included. There is little likelihood that recollection of the original roentgenograms among the 500 read in the test played an important part in the results from these two readers. About a year had passed between the choice of the roentgenograms and the test readings; the readings were made very quickly and, in any case, experience shows that recollection does not play a significant part in determining decisions.

[5] Sometimes, results are reported in the literature as the percentage with correct diagnosis in the total series of tests made, instead of separately for the "true positives" and the "true negatives." Such over-all percentages may easily be misleading, for they depend on the proportion of true positives in the total series. If a series examined contains 1 per cent of positives, a reader who *always* says "negative" will have a score of 99 per cent correct!

Results

The results for the reading of single roentgenograms are shown in table 1. It may be seen that as many as 29 (29.6 per cent) of the 98 positive roentgenograms were read as negative by one reader; the lowest number of false negatives was 12 (12.2 per cent); and the mean number for the five readers was 18.1 (18.5 per cent). With respect to false positives, of 402 negative roentgenograms, one reader designated 49 (12.2 per cent) positive; the lowest number of false positives was two (0.5 per cent), and the average number was 13.7 (3.4 per cent). It may be noted that each of the four readers whose percentage of false negatives was above the average (*B*, *C*, *E*, *G*) had a percentage of false positives below the average, reflecting the fact that a tendency to "underread" has, in general, a dual effect: one of producing a relatively high number of false negatives and the other, a relatively low number of false positives. However, a very proficient reader will be below the average with respect to both false negatives and false positives.

The record of incorrect diagnosis of positive and of negative roentgenograms, for the different readers, presents the essential facts with regard to reliability of the roentgenographic readings.[6] However, it does not immediately reflect the differences that will be found if two readers are compared one with the other, and this may be of some interest. If the errors of the different readers are correlated, that is, if roentgenograms falsely called positive or negative are largely the same ones that are misdiagnosed by another reader, there will be close agreement between the diagnoses of the two readers. But if there is little correlation of errors, there will be greater disagreement. The amount of disagreement is shown in table 2, which can be explained in terms of the first line for reader *A*.

[6] Statistical technology provides a large number of indices or coefficients of association for records of incorrect designations of two categories such as are provided in the present examples. These coefficients can be considered as scorings of the combination of the two kinds of error. A learned review of them has recently been published by Goodman and Kruskal. In the opinion of one of the present writers (J.B.), generally such coefficients obscure rather than clarify the essential significance of the results that are conveyed in the simple statement of the frequency of each kind of error. This view has been presented in a paper of the appended references (5).

TABLE 1

False Negatives and False Positives: Reading of Single Roentgenograms

Reader	Roentgenograms Positive (98)		Roentgenograms Negative (402)	
	False Negative		False Positive	
	Number	Per Cent	Number	Per Cent
A	13	13.3	12	3.0
B	29	29.6	4	1.0
C	19	19.4	7	1.7
D	18	18.4	5	1.2
E	22	22.4	10	2.5
F	12	12.2	7	1.7
G	19	19.4	2	0.5
H	18	18.4	27	6.7
I	13	13.3	49	12.2
Mean.....	18.1	18.5	13.7	3.4

This reader called 97 of the 500 roentgenograms examined positive, 85 of which were really positive. Of these 97, reader *B* called 64 positive, reader *C* called 75 positive, *D* called 75 positive, and so forth. In the same way, the record for the positive readings of each of the readers is presented in the successive lines of the table. The largest disagreement recorded here is for the positive readings of reader *I* who recorded 134 positive diagnoses out of the 500 roentgenograms surveyed; and, of these, reader *B* called only 62 positive. A similar tabulation of disagreement between readers is shown in table 3, in which the disagreement is recorded for the negative diagnoses of each reader. Here the largest disagreement is shown between reader *B* and reader *I*. Reader *B* called 427 of the 500 roentgenograms negative and, of these, reader *I* called only 355 negative.

In table 4, the reliability of the readings made stereoscopically is presented in the same manner as was done with the readings of single roentgenograms, that is, separately for positive and for negative roentgenograms. The best score for false negatives was 5.1 per cent, the worst was 23.5 per cent, and the mean was 14.9 per cent. For false positives, the best score was 0.5 per cent, the worst was 13.2 per cent, and the mean was 2.5 per cent.

In table 5, the scores for readings of single roentgenograms are compared with those for stereoscopic reading. With respect to the positive roentgenograms, it may be seen that the per-

TABLE 2

AGREEMENT AMONG READERS: SINGLE ROENTGENOGRAMS READ POSITIVE

Reader	Called Positive	Called Positive by									
		Actual	A	B	C	D	E	F	G	H	I
A	97	85	—	64	75	75	72	81	74	81	80
B	73	69	64	—	62	60	60	62	63	61	62
C	86	79	75	62	—	73	72	80	69	72	77
D	85	80	75	60	73	—	70	78	68	73	76
E	86	76	72	60	72	70	—	76	64	71	74
F	93	86	81	62	80	78	76	—	72	74	81
G	81	79	74	63	69	68	64	72	—	69	72
H	107	80	81	61	72	73	71	74	69	—	83
I	134	85	80	62	77	76	74	81	72	83	—

TABLE 3

AGREEMENT AMONG READERS: SINGLE ROENTGENOGRAMS READ NEGATIVE

Reader	Called Negative	Called Negative by									
		Actual	A	B	C	D	E	F	G	H	I
A	403	390	—	394	392	393	389	391	396	377	349
B	427	398	394	—	403	402	401	396	409	381	355
C	414	395	392	403	—	402	400	401	402	379	357
D	415	397	393	402	402	—	399	400	402	381	357
E	414	392	389	401	400	399	—	397	397	378	354
F	407	395	391	396	401	400	397	—	398	374	354
G	419	400	396	409	402	402	397	398	—	381	357
H	393	375	377	381	379	381	378	374	381	—	342
I	366	353	349	355	357	357	354	354	357	342	—

centage of false negatives was less with stereoscopic than with single roentgenograms in the case of four of the nine readers, the same in three, and greater in two. It is notable that the reader with the lowest percentage of false negatives using the single roentgenogram (*F*) improved his readings when the stereoscopic method was used; also the reader with the highest percentage of false negatives with the single roentgenogram (*B*) improved his readings, and neither of these improvements was at the expense of an increase in the percentage of false positives. The mean percentage of false negatives was 18.5 per cent with the single roentgenogram and 14.9 per cent with the stereoscopic film, a decrease of 3.6 in percentage of false negatives. With negative roentgenograms, there were fewer false positives with the use of stereoscopic reading with seven of the nine readers, no change with one, and slightly more with one. The mean percentage of false positives was 3.4 with single roentgenograms and 2.5 with stereoscopic films, a decrease of 0.9 in percentage of false positives. Unlike the experience with an attempt to improve

TABLE 4

FALSE NEGATIVES AND FALSE POSITIVES: STEREOSCOPIC READINGS

Reader	Roentgenograms Positive (98)		Roentgenograms Negative (402)	
	False Negative		False Positive	
	Number	Per Cent	Number	Per Cent
A	14	14.3	10	2.5
B	8	8.2	2	0.5
C	19	19.4	1	0.2
D	18	18.4	3	0.7
E	22	22.4	3	0.7
F	5	5.1	3	0.7
G	11	11.2	2	0.5
H	23	23.5	14	3.5
I	11	11.2	53	13.2
Mean	14.6	14.9	10.1	2.5

TABLE 5

COMPARISON OF READINGS OF SINGLE AND STEREOSCOPIC ROENTGENOGRAMS

Reader	False Negative, Per Cent			False Positive, Per Cent		
	Reading, Roentgenogram		Difference	Reading, Roentgenogram		Difference
	Single	Stereoscopic		Single	Stereoscopic	
A	13.3	14.3	+1.0	3.0	2.5	−0.5
B	29.6	8.2	−21.4	1.0	0.5	−0.5
C	19.4	19.4	0	1.7	0.2	−1.5
D	18.4	18.4	0	1.2	0.7	−0.5
E	22.4	22.4	0	2.5	0.7	−1.8
F	12.2	5.1	−7.1	1.7	0.7	−1.0
G	19.4	11.2	−8.2	0.5	0.5	0
H	18.4	23.5	+5.1	6.7	3.5	−3.2
I	13.3	11.2	−2.1	12.2	13.2	+1.0
Mean.	18.5	14.9	−3.6	3.4	2.5	−0.9

the reliability of interpretation by dual reading, in which a decrease in the designation of false negatives was associated with an increase of reading of false positives (3, 4), the use of stereoscopic reading resulted, on the average, in fewer false negatives and also fewer false positives. On the basis of the present investigation, therefore, the use of stereoscopic roentgenograms results, on the average, in an unequivocal improvement over reading with single roentgenograms.

SUMMARY

The reliability of readings of roentgenograms of the thorax was examined on the basis of roentgenograms successively viewed in the manner carried out with patients referred for roentgenographic study in the course of medical examination. Five hundred roentgenograms were presented in random arrangement to each of nine readers, five of whom were roentgenologists and four, specialists in diseases of the thorax. Ninety-eight of the roentgenograms had previously been designated as "positive" and 402 as "negative." With single roentgenogram readings, the average was 18.5 per cent for "false negatives" and 3.4 per cent for "false positives." This variability is in the same order of magnitude as that found in previous investigations of similar roentgenographic examinations.

The same roentgenograms were presented in a new random order, in corresponding pairs, and read as stereoscopic roentgenograms. There was an improvement in the average for both the false negatives, (which was reduced from 18.5 per cent to 14.9 per cent) and the false positives (which was reduced from 3.4 per cent to 2.5 per cent) (table 5). It is considered that these results show that the use of stereoscopic reading has a definite advantage, in improvement of reliability, over the use of single-roentgenogram reading.

SUMARIO

Identificación de las "Positivas" en las Lecturas Radiográficas

Se investigó la fidedignidad de las lecturas de las radiografías torácicas, tomando por base las estudiadas sucesivamente en la forma que se hace esto en los enfermos enviados para estudio radiográfico en el transcurso del examen médico. Se presentaron, dispuestas al azar, 500 radiografías a cada uno de nueve lectores, cinco de los cuales eran radiólogos y cuatro especialistas en enfermedades del tórax. Noventa y ocho de las radiografías habían sido antes designadas como "positivas" y 402 como "negativas." Con lecturas únicas de las radiografías, el promedio fué de 18.5 por ciento para "seudonegativas," y de 3.4 por ciento para "seudopositivas." Esta variabilidad corresponde al mismo género de magnitud ya observado en previas investigaciones de semejantes exámenes radiográficos.

Se presentaron las mismas radiografías, en un nuevo orden al azar, en pares correspondientes, leyéndose como radiografías estereoscópicas. Hubo una mejoría de los promedios tanto para las seudonegativas (que bajaron de 18.5 por ciento a 14.9 por ciento) como para las seudopositivas (que bajaron de 3.4 por ciento a 2.5 por ciento). Considérase que estos resultados demuestran que el uso de la lectura estereoscópica posee una ventaja bien definida, en el aumento de la exactitud, comparado con el uso de una lectura única de la radiografía.

RESUME

Identification de "positifs" dans l'interprétation radiographique

La fidèlité de la lecture de radiographies du thorax est considérée en partant de radiographies vues à la suite l'une de l'autre comme c'est pratiqué pour les malades envoyés pour examens radiographiques au cours d'un examen médical. Cinq cent radiographies furent présentées sans ordre préétabli, à chacun des neuf interprètes, cinq d'entr'eux étant des radiologues et quatre, des

spécialistes des maladies du thorax. Quatre vingt dix huit radiographies avaient été auparavant etiquetées "positives" et quatre cent deux "négatives." Avec une seule lecture radiographique, la moyenne de "fausses negatives" fut de 18,5% et de 3,4% pour les "fauses positives." La variabilité du même ordre de grandeur que celle trouvée lors d'investigations préalables sur des examens radiographiques semblables.

Les mêmes radiographies furent présentées dans un nouvel ordre dispersé, par paires correspondantes, et lues comme en stéréoscopie. Il y eut une amélioration de la moyenne pour les fausses négative (qui furent réduites de 18,5% à 14,9%) et des fausses positives (qui furent réduites de 3,4% à 2,5%) (table 5). On considère que ces résultats montrent que l'emploi de la lecture stéréoscopique a un avantage net sur la simple lecture des radiographies en étant d'une plus grande fidèlité.

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AVIAN TUBERCLE BACILLI AND OTHER MYCOBACTERIA[1,2]

Their Significance in the Eradication of Bovine Tuberculosis

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Introduction

In the United States the campaign to eradicate bovine tuberculosis began officially in 1917. During the more than forty years the program has been in effect impressive gains have been recorded. However, despite the loudly acclaimed triumphs, based on the winning and retention of accreditation by all of the states of the Nation, certain facts about the program are cause for grave concern.

A review and an appraisal of the eradication program from its beginning to the present reveal a critical situation. Many believed that the program as originally announced meant that tuberculosis of cattle would in truth be exterminated. Instead, and particularly during the past fifteen years, the effectiveness of the program has lessened appreciably.[3]

It is time to realize that, unless the program of eradication, based on sound concepts of control of infectious disease, is faithfully adhered to, final triumph over bovine tuberculosis is not likely to be achieved.

Fortunately, those of the U. S. Department of Agriculture who now have the responsibility for completing the tuberculosis eradication program are fully aware of the seriousness of the situation. They have initiated dynamic means to obtain and to disseminate more information, and they seem determined to attack the problems that remain with vigor, enthusiasm, and intelligence. This is reassuring.

The most important—I would say, the indispensable—weapon in the attack on bovine tuberculosis has been and will continue to be the tuberculin test. To administer and to interpret this test correctly require more than mechanical perfection. Since 1908, when the test was first described by Moussu and Mantoux (3), experience has established the shortcomings as well as the merits of the procedure.

The merits and practical usefulness of the test are reflected in the impressive nationwide decrease in the incidence of bovine tuberculosis. The shortcomings are concerned principally with the qualitative aspects of the antigen. By this is meant the ability of tuberculin prepared from mammalian tubercle bacilli to elicit a significant dermal reaction to hypersensitivity induced previously by avian tubercle bacilli or perhaps by other mycobacteria, some of which may be unclassified.

It is my intention, in the text that follows, to present information and comment pertaining to the several facets of the subject implied by the title. Considered will be natural infections of bovine and other animals with avian tubercle bacilli, and the ability of other types of tubercle bacilli to infect heterologous hosts. The question of reciprocal or cross reactions to tuberculins will be examined, as will the possibility that certain unclassified mycobacteria may induce hypersensitivity to diagnostic doses of tuberculin. Last, the significance of mycobacteria other than bovine tubercle bacilli in the eradication of bovine tuberculosis will be discussed.

Tuberculosis is world-wide in distribution. Commonly known, frequently ignored, but of much interest and practical importance, is the occurrence of the disease in a wide variety of species. Tuberculosis occurs naturally in most of the warm-blooded mammals and fowl, and in some cold-blooded animals, including alligators, fish, turtles, snakes, frogs, and iguanas (4–7). This distribution indicates that few species of animals have an absolute resistance to the disease. To stress this point further, only recently I learned of a mycobacterial pathogen of which the natural host is a variety of fresh-water snails (8).

The formidable nature of tuberculous infections

[1] From the Department of Medicine and Surgery, Veterans Administration, Washington, D. C.

[2] Presented at the Conference on Eradication of Bovine Tuberculosis, sponsored by the U. S. Department of Agriculture and the Division of Livestock Sanitation, Madison, Wisconsin, January 5–8, 1959.

[3] Additional information regarding the eradication of bovine tuberculosis has been contributed by Steele and Ranney (1) and by Francis (2).

in warm-blooded animals becomes particularly evident when the several forms of tubercle bacilli are recalled: the human, the bovine, the avian, and the vole. None of these is strictly host-specific. Instead, each is capable of naturally infecting several heterologous hosts (7, 9, 10).

Natural Mycobacterial Infections

Bovine tubercle bacillus: In addition to infecting bovine animals, the bovine tubercle bacillus may, and often does, produce tuberculosis in human beings, swine, dogs, and cats. Most birds, including domestic barnyard fowl, are highly resistant to infection by this organism; but paradoxically, the bacillus may infect canaries and parrots. Of paramount importance is the well-established fact that human beings are susceptible to infection by the microorganism of bovine tuberculosis. In man it produces tuberculous lesions in bones and joints, and is fully capable of producing pulmonary tuberculosis as severe as similar pulmonary infections caused by the human form of the organism (10).

Reports indicate that bovine tubercle bacilli can be passed successfully from human beings to cattle.

An impressive instance was reported in 1954 by Tice of New York State (11). Despite repeated tuberculin tests, and the removal of all reactors, and the strict maintenance of sanitation, infection continued to occur in new additions to the herd. It was not until three complete herds had been slaughtered because of positive tuberculin reactions that the owner was suspected as the possible source of the infection. Subsequent investigation revealed that he had been a patient at a tuberculosis hospital for a few weeks just prior to the first tuberculin testing of his dairy herd. Studies of both the sputum from the owner and of the infected lymph nodes of his cattle that had reacted to tuberculin revealed tubercle bacilli of the bovine variety. Tice's study established that the owner had contracted pulmonary tuberculosis from his original herd and subsequently had infected the three new replacement herds within a period of two and a half years.

Tice's report emphasizes the importance of medical examination of persons who have access to dairy premises. Obviously, those persons who have active pulmonary tuberculosis should be excluded from premises frequented by cattle. The possibility of natural transmission of human tubercle bacilli from man to cattle is remote, but there is a greater possibility that, by natural exposure to human tubercle bacilli, cattle may become sensitized to mammalian tuberculin. Especially significant would be the presence in the herd of tuberculosis of obscure origin, as revealed by repeated testing with tuberculin.

Human tubercle bacillus: The human tubercle bacillus, the etiologic agent of most tuberculous infections in man, is capable of producing serious disease in heterologous hosts, including parrots, monkeys, swine, dogs, and rarely cattle.[4] In cattle, infection with human tubercle bacilli usually remains localized, and the lesions show a tendency to heal or to become quiescent.

In a review made several years ago reports of nine cases were found in which the infectious agent in cattle was established as the human tubercle bacillus (7). In this small series the demonstrable lesions in most instances occurred in the mesenteric, the mediastinal, or the bronchial lymph nodes; significant lesions in the parenchymal organs were not observed. In the one additional case reported by Moses and myself, lesions of tuberculosis in an adult bovine consisted of a small, encapsulated caseocalcareous nodule in the parenchyma of one lung, a caseocalcareous lesion in a bronchial lymph node, and a lesion of similar character in one of the mesenteric nodes (7). Tubercle bacilli were obtained with the cultural and pathogenic characteristics of the human form from the lesions of the lung and the bronchial lymph node.

A résumé of the evidence suggests the infrequency of the infection of cattle with human tubercle bacilli. The native resistance of cattle and the character and anatomic distribution of the lesions make it unlikely that this type of tuberculous infection could be transmitted from one animal to another. Although the possibility exists, there is no evidence that a bovine animal infected with human tubercle bacilli will eliminate the infective bacteria with the milk.

There is one phase of this problem that justifies emphasis. This pertains to the sensitization of cattle to mammalian tuberculin as a consequence of exposure to human tubercle bacilli derived from tuberculous human beings. Evidence has accumulated that this may occur. It is of interest to note that, after the human source of the infection has been removed, the animal previously

[4] The question of natural infection of cattle with human tubercle bacilli has been reviewed previously (2, 7, 10).

hypersensitive to tuberculin gradually loses its sensitivity to this antigen.

Although tuberculosis of man caused by human tubercle bacilli can be transmitted to cattle, the consequences of such transmission must be viewed with circumspection. In so-called problem herds, in which the recurrence of reactions to tuberculin cannot be explained by proved infections with bovine tubercle bacilli, the possibility of exposure to human tubercle bacilli should not be ignored.

Avian tubercle bacillus: Few bacterial agents are pathogenic for as many different species of animals as is the avian tubercle bacillus. In addition to being virulent for most of the wild and domesticated birds, this microorganism is capable of producing infections of variable severity in a considerable number of different species of mammals (12). Factors that constitute abundant opportunities for the transmission of avian tuberculosis to susceptible hosts—homologous as well as heterologous—are: the widespread distribution of the disease among domestic fowl in the northern hemisphere; the great number of tubercle bacilli present in the lesions of infected chickens; and the frequent presence in tuberculous chickens of ulcerated intestinal lesions from which large numbers of viable, virulent avian tubercle bacilli are discharged with feces.

How frequently avian tubercle bacilli infect cattle is not known, even approximately. In areas where tuberculosis commonly affects chickens, transmission of the avian infection to cattle and swine, and perhaps to sheep, probably is far more frequent than reported instances suggest. It is well to remember that in the bovine animal natural infection with avian tubercle bacilli usually results in localized, relatively nonprogressive, benign lesions which frequently are small and which may be difficult to find. Widespread progressive destructive morbid changes which often characterize infection of cattle with bovine tubercle bacilli are seldom observed in cattle when the infection is caused by avian tubercle bacilli. However, there are exceptions of which the following two cases will serve as examples:

The first case was reported in 1954 by Drs. Fincher, Evans, and Saunders of the New York State Veterinary College (13).

The animal was a 3-year-old Guernsey cow. The significant symptoms were paresis and a persistent vaginal discharge. Because of the paresis the animal was killed. Lesions of tuberculosis were found in the cerebral and spinal meninges and in the uterus. Avian tubercle bacilli were isolated from the uterus. The identity of the organism was established by tests of pathogenicity. According to the writers, the most likely source of the infection was a meadow that the animal had occupied for approximately thirty days, four months prior to the onset of symptoms. Significantly, the meadow had been fertilized with manure from a poultry farm. Tuberculin tests were done on most of the 34 cattle that had occupied the same meadow as the animal in which tuberculosis occurred. None reacted to mammalian tuberculin. To avian tuberculin, 9 had what were reported as "fair reactions" and 8 had reactions that were considered "slight."

The second case was made available to me through the kindness of Drs. Charles L. Davis and Wayne A. Anderson, both of Denver (14). This case has not been published; the report is based on data from the files of the Denver Laboratory, Animal Disease and Parasite Research Division, U. S. Department of Agriculture.

During routine post-mortem examination of the carcass of a 7-year-old cow in a federally inspected establishment at Spokane, Washington, lesions of a miliary character were observed in the liver, kidneys, and lungs. The cervical and thoracic lymph nodes were enlarged, but without evidence of necrosis or nodular foci. The spleen was slightly enlarged and was described as pulpy. Portions of the liver, kidney, and lymph nodes were forwarded to the Denver laboratory for examination and study. Tuberculosis was first suspected on histologic examination of the lesions, and was subsequently confirmed by culture of tissues from the liver. By animal tests of pathogenicity the organism proved to be an avian tubercle bacillus. It was markedly virulent for chickens, but it was not virulent for guinea pigs.

Dr. Harold E. Moses of Purdue University has informed me of two herds of cattle in which animals were proved to be infected with avian tubercle bacilli (15).

The first herd consisted of 250 to 300 Holstein cattle. Records of eleven tests, from 1954 to 1958, inclusive, disclosed that a total of 49 animals had reacted to tuberculin. At necropsy of 36 of these animals, so-called skin lesions were found in 3. A solitary caseocalcareous lesion in a mesenteric lymph node was found in one, and a lesion was present in a portal lymph node of another. A culture was obtained from the mesenteric lesion

that had the cultural and pathogenic features of avian tubercle bacilli.

The second herd consisted of 40 Guernsey cattle. Tuberculin reactions were first observed after testing in the spring of 1958. Seventeen of the animals were considered to be reactors. The same animals were retested about three months later, with the concomitant use of mammalian tuberculin and johnin. Fourteen of the previous reactors reacted to both antigens, but the reactions to the johnin were more intense than the reactions to the tuberculin. The reacting animals were removed from the herd and slaughtered at a federally supervised abattoir. Tuberculous lesions were found in a bronchial lymph node of one animal and in submaxillary and mesenteric lymph nodes of another. From these lesions avian tubercle bacilli, identified by tests of pathogenicity, were obtained. No evidence of Johne's disease was found in any of the animals.

Another example of infection by avian tubercle bacilli in a heterologous host was reported in 1957 by Hall and Winkel of the Animal Disease Diagnostic Laboratory at Madison, Wisconsin (16).

During the 1955–1956 season a mink rancher had lost a considerable number of animals, far in excess of the mortality in previous years. No significant symptoms were noted; however, some of the animals were lethargic and emaciated. The most striking change noted at necropsy was the marked lymphadenopathy. On the basis of the results of animal inoculation and the characteristics of the cultures it was concluded that the infection in the minks examined was caused by avian tubercle bacilli.

A possible source of the infection in the mink was a shipment of swine livers that were considered inedible for human beings, but which were fed to the minks several weeks prior to the onset of visible illness.

On the basis of my experience with avian tuberculous infections in swine, it is possible that lesions of avian tuberculosis were present in the swine livers. In so far as I know, this is the first instance in which susceptibility of mink to avian tubercle bacilli has been established. Tuberculosis in mink caused by bovine tubercle bacilli has been recognized previously (2). It would be interesting to know if mink are likewise susceptible to the bacilli of human tuberculosis.

The sensitivity to avian tuberculin of human beings with proved clinical tuberculosis and of apparently healthy or presumably nontuberculous persons has intrigued many investigators (12, 17). The observations may be summarized as follows:

1. Persons infected with either human or bovine tubercle bacilli may react positively to both mammalian and avian tuberculins.

2. Why some persons have a hypersensitivity to avian tuberculin and others do not is not known. Sensitivity of man to avian tuberculin does not necessarily indicate infection with avian tubercle bacilli. Instead, the reaction may represent a reciprocal or group sensitization as a consequence of the presence in the tissues of other and perhaps unclassified mycobacteria.

3. Most persons who react positively to mammalian tuberculin will react also, but less intensively, to avian tuberculin.

4. Limited observations suggest that persons with calcified pulmonary lesions react to avian PPD, although some of these persons fail to react to human PPD.

5. In persons in whom the reaction to avian tuberculin is definitely positive and no reaction to mammalian tuberculin occurs, the presence of infection with avian tubercle bacilli should be considered. However, in such instances it is not justifiable to conclude on the basis of a positive reaction to avian tuberculin that an avian type of infection actually is present. Such a conclusion can be made only by obtaining from the patient mycobacteria that are proved subsequently to be avian tubercle bacilli.

So-called skin tuberculosis of cattle: This granulomatous condition in cattle, described by Traum (18), is commonly referred to as "skin lesions." The disease is widespread, occurring in the United States and in Western Europe, and the United Kingdom. In the majority of instances the condition has been noted in tuberculosis-free herds and, in so far as is known, the disease is limited to bovine animals.

The lesions vary in size. Some are very small and are revealed only after careful search. The lesions may consist of a single nodule or as many as 20 nodules in the same animal. Occasionally the lesions involve the corium of the skin, and may ulcerate. In the majority of instances the lesions occur subcutaneously. Sites of predilection are the lateral side of the foreleg, the ventral aspect of the abdomen, and the lateral side of the tarsal joint. Less frequently the lesions occur on the neck or shoulders. It is

assumed that the causative agent enters the skin by way of an abrasion. If conditions for pathogenesis are favorable, a slowly progressive granulomatous reaction results.

The tuberculoid character of the majority of the lesions, the presence of alcohol-fast and acid-fast bacillary cells, and the fact that the affected animals frequently react to tuberculin suggest an infection similar in many respects to those produced by tubercle bacilli. Although mycobacteria frequently are numerous in the lesions, artificial cultivation has not been accomplished and attempts to produce the disease experimentally generally have failed.[5]

Traum was the first to observe that lesions of this character sometimes cause cattle to react to tuberculin (18). Sensitivity to tuberculin of animals with this disease is another example of reciprocal sensitization to tuberculin by different species of Mycobacterium. It is well to keep in mind that sensitivity to tuberculin is not necessarily indicative of infection with tubercle bacilli.

Significance of Unclassified Mycobacteria

In attempting to understand the tuberculin hypersensitivity of animals, including man, in which definite exposure to avian, bovine, or human tubercle bacilli is unlikely or at least cannot be proved, consideration should be given to certain unclassified or unidentified mycobacteria. Mycobacteria in this category frequently are obtained from pulmonary exudates and from resected lung tissues of human beings (20). The pathogenic significance of these microorganisms is not always apparent. In some instances, however, these unclassified mycobacteria appear to be related to the patient's illness. There is evidence that unidentified mycobacteria occur also in tissues of animals other than man.

Some years ago Karlson and I obtained from the tonsils of swine fairly rapidly growing, nonchromogenic, acid-fast bacillary organisms that were considered to be unlike previously described mycobacteria (21). These organisms were obtained from approximately 25 per cent of 94 tonsils representing 94 swine. The organisms failed to produce demonstrable disease in chickens, mice, or calves. Likewise, little if any virulence was noted when the organisms were introduced into guinea pigs and rabbits. The organisms did, however, sensitize guinea pigs to avian tuberculin and to homologous culture filtrates; sensitivity to mammalian tuberculin did not occur.

Recently, H. Williams Smith of England searched for mycobacteria in retropharyngeal and ileocecal lymph nodes and in spleens of 100 apparently healthy cattle, and he cultured several unidentified strains of acid-fast bacilli (22, 23). Smith's observations suggest that the apparently normal-appearing lymph nodes of the alimentary tract of animals may harbor mycobacteria unlike the presently recognized and classified species. This should be a profitable area for future investigations.

What significance the unclassified mycobacteria may have in the ecology of natural and heterologous hosts is unknown. Although their potential for pathogenesis may be negligible, their capacity to induce a state of hypersensitivity to tuberculin is worthy of consideration.

General Comment

It should be emphasized that a significant relationship exists between tuberculosis of poultry and the prospects of eventually eradicating tuberculosis in cattle. It seems logical that, after natural exposure to avian tuberculous infections, cattle may develop hypersensitivity to avian tuberculin. Furthermore, evidence indicates that probably a third of the cattle sensitized by avian tubercle bacilli will react positively to mammalian tuberculin. Although available facts are meager, there is reason to believe that in the United States and perhaps in the United Kingdom avian tubercle bacilli sensitize more cattle to mammalian tuberculin than is generally recognized. It is likely that sensitivity to tuberculin of at least some of the so-called nonmacroscopic lesion reactors is caused by exposure of the cattle to avian tubercle bacilli. It is obvious that, unless tuberculosis of chickens and other barnyard fowl is eradicated, cattle will continue to be infected with avian tubercle bacilli, and some will react to tuberculin. As a

[5] Hedstrom (19) of Stockholm reported the successful reproduction of the disease in 3 cows inoculated intracutaneously with composite material from four specimens. The lesions in the experimentally infected animals were similar to those occurring naturally, and the animals became sensitized to bovine and to avian tuberculin.

result, valuable animals will be removed from the herd and condemned to slaughter, even though the last animal infected with proved bovine tuberculosis may have been detected and disposed of years previously.

It is obvious that eradication of tuberculosis, whether in man or in animals, can be achieved only after the detection of all infected hosts. Persons with active tuberculosis should be isolated; animals found to be infected must be destroyed; and means should be employed to prevent the resurgence of the disease from obscure or hidden sources. The possible existence of latent avian tuberculous infections in swine, sheep, and other species should be kept in mind. Latent infections may become activated and provide sources of contagion to other susceptible animals. Furthermore, we must remain alert to the possibility that mycobacteria other than those presently recognized and classified may lodge in tissues, particularly lymphoid tissues, and slowly multiply. Eventually they may induce a sensitivity to tuberculin, even though tissue changes indicative of pathogenesis may not be demonstrable. One of the most urgent and potentially fruitful problems for research in tuberculosis of man and of animals is that embracing the reciprocal relationship of the tuberclin reaction to hypersensitivity induced by mycobacteria other than true tubercle bacilli.[6]

With few exceptions, the diseases produced naturally by mycobacteria can be classified properly as infectious granulomas. This designation is often fortunate and convenient for the hard-pressed pathologist who cannot always determine by examination of the morbid tissue the precise nature of the causative agent. Structural changes or patterns in the tissue that represent the host's response to mycobacteria and to many of the fungous infections have several cellular components in common. The diagnosis of such material based on histopathology alone is always unwise and often difficult, if not impossible. In dealing with infectious granulomas, the pathologist must recognize the diagnostic limitations of tissue microscopy. This is particularly important because the causative agent may be present in small numbers or may be difficult to recognize unless suitable bacteriologic studies are done.

The effective attack against tuberculosis in man and in animals is dependent upon precise diagnosis. The medical bacteriologist contributes significantly to the definitive diagnosis of infections of obscure character. However, he must be aware of the pitfalls inherent in the assumption that the diagnosis of tuberculosis and other mycobacterial diseases can be established unequivocally by the isolation of acid-fast bacilli *in vitro*.

It has become increasingly evident that features other than the tinctorial characteristics of the organism are essential in distinguishing commonly known mycobacteria, such as tubercle bacilli, from other members of the same genus. It is noteworthy that, as the role of the bacteriology laboratory has expanded in the diagnosis of mycobacterial and fungous diseases, the frequency of finding infections presumably caused by unclassified or unidentified mycobacteria has increased.

Saprophytic mycobacteria often occur in certain clinical materials, and evidence is accumulating that morbid tissues may harbor potentially pathogenic mycobacteria which taxonomically are not identified. The importance of these bacteria in the induction of hypersensitivity to tuberculin in man and in animals should not be minimized. Instead, the presence of such bacteria in clinical materials, animal or human, should be considered a challenging opportunity to establish new and urgently needed facts necessary to resolve the many enigmas associated with the vast group of microorganisms which constitute the genus Mycobacterium.

Summary

In the preceding text the objective has been to emphasize the existence of a significant relationship between certain mycobacteria, other than human or bovine forms of tubercle bacilli, and the prospects of eventually eradicating tuberculosis in cattle. The importance of the tuberculin test as the indispensable weapon in the attack on bovine tuberculosis is reiterated. In addition, certain serious shortcomings of the procedure are recounted, particularly those concerned with the qualitative aspect of the

[6] Additional information concerning the specificity of the state of hypersensitivity in natural and in experimental infections with various species or types of mycobacteria may be found in the admirable review by Doull and Strachan (24).

antigen. Natural infections of cattle and other mammals with the organism of avian tuberculosis are considered, as is the ability of other types of tubercle bacilli to infect heterologous hosts. The question of reciprocal or cross reactions of tuberculin is also emphasized. Attention is drawn to the possibility that certain unclassified mycobacteria may also induce hypersensitivity to diagnostic doses of tuberculin. Finally, it is concluded that eradication of tuberculosis, whether in man or in animals, can be achieved only after the detection of all infected individuals. The recognition generally that hypersensitivity to tuberculin may be induced by, or be associated with, mycobacterial infections other than the classified forms of the genus Mycobacterium would contribute much to a more comprehensive and definitive understanding needed to achieve the eventual eradication and control of the still unconquered mycobacterial diseases.

Sumario

Los Bacilos Tuberculosos Aviarios y Otras Microbacterias

En el ensayo precedente, el objetivo ha consistido en recalcar la existencia de una relación importante entre ciertas microbacterias, distintas de las formas humanas o bovinas de los bacilos tuberculosos, y las probabilidades de erradicar con el tiempo la tuberculosis bovina. Reitérase la importancia que posee la prueba de la tuberculina como arma indispensable para el ataque contra la tuberculosis bovina. Además, señálanse ciertas graves deficiencias del procedimiento, en particular los relativos al aspecto cualitativo del antígeno. Se consideran las infecciones naturales del ganado bovino y de otros mamíferos por el microbio de la tuberculosis aviaria y también la capacidad de otras formas del bacilo tuberculoso para infectar huéspedes heterólogos. Hácese además hincapié en el punto de las reacciones recíprocas o cruzadas de la tuberculina. Llámase la atención sobre la posibilidad de que ciertas micobacterias inclasificadas provoquen también hipersensibilidad a dosis diagnósticas de tuberculina. Por fin, se deduce que la erradicación de la tuberculosis, ya en el hombre o los animales, sólo puede lograrse después del descubrimiento de todos los infectados. El reconocimiento general de que la hipersensibilidad a la tuberculina puede ser provocada por, o estar asociada con, infecciones micobacterianas distintas de las producidas por las formas clasificadas del género *Mycobacterium* contribuiría mucho a una comprensión más amplia y definitiva, como la que se necesita para obtener la erradicación y el dominio a la larga de las afecciones micobacterianas todavía inconquistadas.

Resume

Bacilles tuberculeux aviaires et autres mycobactéries

Dans l'examen précedent, l'objectif était d'insister sur l'existence d'une relation notable entre certaines mycobactéries, autres que les formes humaines et bovines, et les projets pour enrayer éventuellement la tuberculose du bétail. L'importance de la réaction à la tuberculine en tant qu'arme indispensable dans l'attaque contre la tuberculose bovine est à nouveau signalée. En plus certaines erreures sérieuses de la méthode sont notées, particulièrement celle en rapport avec la qualité de l'antigène. Les infections naturelles du bétail et autres mammifères avec des bacilles tuberculeux du type aviaire sont considéreées ainsi que la possibilité d'autres types de bacilles tuberculeux d'infecter des hôtes différents. La question de réciprocité et de cross-réactions vis à vis de la tuberculine est aussi soulignée. L'auteur attire l'attention sur la possibilité qu'ont aussi certaines mycobactéries non classées de déclencher une hypersensibilité avec des doses de tuberculine infimes. Finalement l'auteur conclut qu'enrayer la tuberculose, celle de l'homme et des animaux ne peut être obtenu quaprès avoir dépisté toutes les personnes infectées. La connaissance générale que l'hypersensibilité à la tuberculine peut être causée par des infections mycobactériennes—ou associée à leur présence—ces mycobactéries étant autres que les formes classées du genre mycobactéries, contribuerait largement à une compréhension plus étendue et plus précise qui est nécessaire pour achever d'enrayer éventuellement et de contrôler les maladies non vaincues dues aux mycobactéries.

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BACTERIOPHAGE SUSCEPTIBILITY AND TUBERCULIN SPECIFICITY OF UNCLASSIFIED MYCOBACTERIA[1, 2]

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INTRODUCTION

The unclassified mycobacteria which are associated with pulmonary diseases closely resembling tuberculosis have recently received increasing attention and have been intensively studied from various standpoints.

Bacteriophages active against various types of mycobacteria have been isolated by Froman and associates (1) and Takeya and Yoshimura (2). The phage-susceptibility pattern of various mycobacteria, except unclassified mycobacteria, to the isolated phages were also reported in detail (3–5). These experiments revealed that typical virulent avian tubercle bacilli are resistant to lysis by all of the phages used in their experiments and that phage-typing can be used as a tool in the screening of possible avian tubercle bacilli (4). The tuberculin specificity of unclassified mycobacteria was reported in an early study by Tarshis and Frisch (6) using crude tuberculin. Purified tuberculin proteins are known to be more useful in differentiating sensitizations with various types of mycobacteria by means of cross-tuberculin reaction (7–10). Consequently, further specificity studies using purified tuberculin proteins prepared from unclassified mycobacteria will present more exact and detailed information.

The purpose of the present paper is to report the phage susceptibility, the tuberculin specificity (determined by purified protein preparations), and the potassium tellurite susceptibility of unclassified mycobacteria.

MATERIALS AND METHODS

Cultures and sources: The unclassified mycobacteria tested were 5 photochromogens, 3 nonchromogens, and one scotochromogen, all of which were isolated in the United States, and 5 unclassified mycobacteria isolated in Japan (table 1). The Matsumoto strain was isolated from multiple tuberculous abscesses (18); the Ishii (19), Yamamoto (20), and Ninomiya (21) strains were repeatedly isolated and/or were from resected lung tissues of patients believed to have tuberculosis; and the Nagata strain (22) was isolated from a cervical lymph node. In addition to these unclassified mycobacteria, strains of mycobacteria isolated from pulmonary or intestinal lymph nodes of autopsied dogs (23, 24) were tested for bacteriophage susceptibility.

The H37Rv and Hori strains of human origin, the Miwa and BCG strains of bovine origin, the A4121, A4110, A71, and Flamingo strains of avian origin, and the *M. 607*, *M. phlei*, and *M. Jikei* of saprophytic mycobacteria were used as controls.

Mycobacteriophage and phage-susceptibility test: Ten strains of mycobacteriophages isolated by Takeya and co-workers (2, 5) were used. They were B-1, B-2, B-3, A-2, A-3, A-4, A-5, A-6, A-7, and C-3. The "spot test" (5) was used for demonstrating phage susceptibility. An 0.3-ml. dispersed suspension of each test organism grown in Dubos Tween®-albumin medium for ten to fourteen days was mixed with 2.5 ml. of 0.7 per cent semi-solid agar medium containing 4 per cent glycerol and 0.04 M calcium chloride. This mixture was layered on an Ogawa's egg medium plate. After the plate was incubated for three to seven days, a loopful of each stock phage suspension containing approximately 10^7 to 10^8 phage particles was spotted on the plate. The plate was again incubated and observed until the growth was visible.

Purification of protein fraction π: Purified protein fraction π (25, 26) was prepared from the culture filtrate of each test organism grown on Sauton medium for eight to ten weeks. The cultures were heated in a steam sterilizer at 100°C. for one hour and filtered for removal of bacillary bodies. A 20 per cent solution of trichloracetic acid was added to the filtrate until its pH reached 2.0. The precipitate was dissolved in water at pH 7.0. Then, by the addition of N/10 hydrochloric acid the heated C protein was precipitated at the isoelectric point, pH 4.8. This procedure was repeated five times. The final precipitate was dissolved in water, dialyzed, and lyophilized.

The culture filtrate was concentrated through ultrafiltration before purification, when the protein content of the culture was too small to obtain enough trichloracetic acid precipitates.

Electrophoresis: Electrophoretic analyses of purified protein π preparations were made by means of *Antweiler* micro-electrophoresis apparatus, using phosphate buffer, pH 7.95, $\mu = 0.144$ at 20°C. The electrophoretic pattern was obtained by the interferometric method.

[1] From the Department of Bacteriology, School of Medicine, Kyushu University, Fukuoka, Japan.

[2] Presented at the 34th Annual Meeting of the Japanese Association for Tuberculosis, Tokyo, April 6–7, 1959.

TABLE 1

THE CULTURES OF UNCLASSIFIED MYCOBACTERIA

Classification	Strain Designation	Sources
Photochromogen	Bostrum D-35	Pollak and Buhler (11–14)
	Forbes 84	
	No. 8	Case 2 of Wolinsky *et al.* (15)
	No. 22	Sunmount package collection of Dr. D. E. Runyon (16)
	M. Kansasii	
Nonchromogen	100616	Crow *et al.* (17)
	121326	
	No. 7	Sunmount package collection of Dr. D. E. Runyon (16)
Scotochromogen	No. 6	
Unclassified	Matsumoto	Toda and Kashiwagi (18)
	Ishii	Someya and Hayashi (19)
	Ninomiya L1	Urabe and Kawai (20–22)
	Yamamoto S	
	Nagata	

Cross-tuberculin reaction: Guinea pigs were sensitized with 6 mg. of heat-killed bacillary bodies of each test organism suspended in 0.6 ml. of liquid paraffin oil by intramuscular infection. About four to eight weeks after, 0.1 ml. of purified protein fraction π of each organism, containing 0.001 mg. of protein, was simultaneously injected intracutaneously in these guinea pigs. The diameters of the indurations were measured and compared forty-eight hours after infection.

Susceptibility test of potassium tellurite: A sterile solution of potassium tellurite was added to Kirchner sy-ser medium. The volume of each tube was 5 ml., and the final concentration of potassium tellurite varied from 1.0 to 0.001 per cent, as shown in table 7. Two tubes of each dilution and two control tubes were inoculated with 0.004 mg. of each test organism grown in Dubos Tween-albumin medium. The tubes were incubated at 37°C., and growth was observed every week.

RESULTS

Bacteriophage susceptibility of unclassified mycobacteria and mycobacteria isolated from dogs: None of the unclassified mycobacteria employed in this experiment was susceptible to any of the mycobacteriophages, as shown in table 2. In this respect, unclassified mycobacteria used in this experiment resemble avian strains.

The results of the phage-susceptibility test of mycobacteria isolated from dogs are shown in table 3. Five strains with R-type colonies were lysed by some of the phages, but the remaining 4 strains, which showed cultural characteristics similar to nonchromogens, were found resistant to lysis by the phages.

Specificity of purified tuberculin protein π obtained from unclassified mycobacteria in tuberculin reaction: The pH of cultures, the yield of bacillary bodies, and the yield of purified protein π are presented in table 4. The pH of cultures of photochromogens are from 6.0 to 7.6, while those of nonchromogens are strongly basic. Nonchromogens are somewhat smaller in the yield of bacillary bodies and markedly smaller in the yield of purified protein π than photochromogens.

Electrophoretic analyses were made on each of the π preparations to determine their protein, carbohydrate, and nucleic acid content. The results obtained are shown in table 5. Each pattern resembles that of protein π obtained from the human strain (26), but the amount of nucleic acid was rather high in the protein π of the unclassified strains.

Guinea pigs sensitized with 10 strains of test organisms were cross-tested with 9 kinds of purified protein π, as shown in table 6. In the animals sensitized with photochromogens and also with nonchromogens, the degree of hypersensitivity to the homologous protein was approximately the same as to the heterologous ones within the same group. This indicates that in mycobacteria of the photochromogen group and in those of the nonchromogen group a marked respective group specificity exists with respect to the tuberculin reaction. The fact that purified protein of H37Rv was capable of producing a considerably stronger reaction in the animals sensitized with the bacteria of the photochromogen group suggests that photochromogens are more closely related to human tubercle bacilli

TABLE 2

BACTERIOPHAGE SUSCEPTIBILITY OF UNCLASSIFIED MYCOBACTERIA

Strain	Bacteriophage									
	B-1	B-2	B-3	A-2	A-3	A-4	A-5	A-6	A-7	C-3
Photochromogen										
Forbes 84	−	−	−	−	−	−	−	−	−	−
Bostrum D-35	−	−	−	−	−	−	−	−	−	−
M. Kansasii	−	−	−	−	−	−	−	−	−	−
No. 8	−	−	−	−	−	−	−	−	−	−
No. 22	−	−	−	−	−	−	−	−	−	−
Nonchromogen										
100616	−	−	−	−	−	−	−	−	−	−
121326	−	−	−	−	−	−	−	−	−	−
No. 7	−	−	−	−	−	−	−	−	−	−
Scotochromogen										
No. 6	−	−	−	−	−	−	−	−	−	−
Unclassified										
Matsumoto	−	−	−	−	−	−	−	−	−	−
Ishii	−	−	−	−	−	−	−	−	−	−
Nagata	−	−	−	−	−	−	−	−	−	−
Ninomiya Ll	−	−	−	−	−	−	−	−	−	−
Yamamoto S	−	−	−	−	−	−	−	−	−	−
Control										
Human strain (H37Rv)	+++	+++	+++	+++	++	−	−	−	−	−
Bovine strain (Miwa)	+++	+++	+++	+	−	−	−	−	−	−
Avian strain (A4110)	−	−	−	−	−	−	−	−	−	−
Saprophytes										
M. phlei	+++	+++	+++	+++	+++	−	−	−	−	
M. smegmatis	+++	+++	+++	+++	−	−	−	−	−	
M. 607	+++	+++	+++	+++	+++	+++	−	+++	+++	

+++: Complete lysis.
++: Lysis with resistant colonies.
+: Growth inhibition at the spotted place or several plaques.
−: No lysis and no growth inhibition.

TABLE 3

BACTERIOPHAGE SUSCEPTIBILITY OF MYCOBACTERIA ISOLATED FROM DOGS

Strain	Type of Colony	Bacteriophage										Pathogenicity for Guinea Pig
		B-1	B-2	B-3	A-2	A-3	A-4	A-5	A-6	A-7	C-3	
Nogata 23	R	+++	++	++	+++	−	−	−	−	−	−	Virulent
Nogata 70	R	+++	+++	++	+	−	−	−	−	−	−	
Kasuya 13	R	++	++	+	+	−	−	−	−	−	−	
Kasuya 23	R	+++	+++	+++	++	++	−	−	−	−	−	
Mizuma 21	R	+++	+++	++	+	−	−	−	−	−	−	
Kasuya 24	S	−	−	−	−	−	−	−	−	−	−	Avirulent or weakly virulent
Mizuma 21-2	S	−	−	−	−	−	−	−	−	−	−	
Ukiwa 1-2	S	−	−	−	−	−	−	−	−	−	−	
Ziei 97	S	−	−	−	−	−	−	−	−	−	−	

+++: Complete lysis.
++: Lysis with resistant colonies.
+: Growth inhibition at the spotted place or several plaques.
−: No lysis and no growth inhibition.

TABLE 4

YIELD OF TUBERCULIN PURIFIED PROTEIN π

Strain	Final pH of Culture Media	Weight of Bacillary Bodies per Liter of Culture Media	Yield of π per Liter of Culture Filtrate
		gm.	*mg.*
Photochromogen			
Bostrum D35	7.6	7.3	390
M. Kansasii	6.6	10.3	600
No. 22	6.0	7.0	740
Nonchromogen			
100616	8.2	6.1	60
121326	9.0	6.3	90
No. 7	8.6	5.5	40

TABLE 5

ELECTROPHORETIC ANALYSES OF THE π PREPARATIONS FROM UNCLASSIFIED MYCOBACTERIA

Strain	Fast-moving Component (Nucleic Acid)	Main Component (Protein)	Slow-moving Component (Polysaccharide)
	per cent	*per cent*	*per cent*
Photochromogen			
Bostrum D35	9.2	79.2	11.6
M. Kansasii	11.0	83.9	5.1
No. 22	4.4	84.0	11.6
Nonchromogen			
100616	7.9	81.5	10.6
121326	10.9	80.3	8.8

The yield of π from the culture filtrate of No. 7 was too small to be examined by electrophoresis.

TABLE 6

CROSS-TUBERCULIN REACTION BY PURIFIED TUBERCULIN PROTEIN π PREPARED FROM UNCLASSIFIED MYCOBACTERIA

Purified Protein Prepared from	Guinea Pigs Sensitized with									
	Human Strain H37Rv	Photochromogen			Nonchromogen			Scoto-chromo-gen No. 6	Avian Strain A71	*M. Phlei*
		Bostrum D-35	*M. Kansasii*	No. 22	100616	121326	No. 7			
Human strain H37Rv	14.2*	10.0	9.0	8.6	9.0	6.6	7.0	4.7	8.3	2.5
Photochromogen										
Bostrum D25	5.0	10.8	12.5	11.0	7.5	7.0	5.8	5.7	7.3	3.0
M. Kansasii	8.5	14.0	14.0	13.0	9.5	9.8	8.6	5.7	7.6	4.0
No. 22	4.2	10.0	10.5	12.2	6.5	4.7	5.8	5.0	4.0	3.0
Nonchromogen										
100616	5.0	8.3	8.0	7.3	12.5	14.3	10.3	10.7	11.2	5.0
121326	5.7	8.7	8.5	6.3	13.5	15.0	10.3	10.7	11.2	3.0
No. 7	7.5	8.8	7.0	8.7	13.0	11.3	15.3	9.0	12.3	3.5
Avian strain A71	5.7	7.0	8.5	7.0	11.5	13.0	16.0	7.3	14.8	4.5
M. phlei	2.5	3.0	2.5	4.0	5.0	6.0	5.0	3.0	4.9	13.5

* Numbers represent mean diameters of indurations in mm. measured forty-eight hours after intracutaneous injections.

TABLE 7

INHIBITORY ACTION OF POTASSIUM TELLURITE FOR MYCOBACTERIA

Concentration of Potassium Tellurite Added to Kirchner Sy-ser Medium	Days after Cultivation	Human Strain		Bovine Strain		Avian Strain			Unclassified Strain								Saprophytic Strain		
		H37Rv	Hori	Miwa	BCG	A4121	A71	Flamingo	Forbes 84	Bostrum D35	*M. Kansasii*	No. 8	No. 22	121326	No. 7	Matsumoto	*M. Smegmatis*	*M. 607*	*M. Kaerujikei*
per cent																			
1.0*	7	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	14	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	28	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−			
0.1	7	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±	+	+
	14	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	++
	28	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−			
0.05	7	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+
	14	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	++	++	++
	28	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−			
0.01	7	−	−	−	−	±	−	−	±	−	−	−	−	−	−	−	++	++	++
	14	−	−	−	−	+	±	+	+	+	−	+	+	±	±	±	++	+++	+++
	28	−	−	±	−	+	+	+	++	+	+	++	++	+	+	−			
0.005	7	−	−	−	−	+	±	±	+	+	−	+	+	±	±	−	+++	+++	+++
	14	−	−	±	−	++	++	++	++	++	+	++	++	+	+	+	+++	+++	+++
	28	−	−	+	±	+++	+++	+++	+++	++	++	++	+++	++	++	++			
0.001	7	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+++	+++	+++
	14	±	±	+	±	+++	+++	+++	+++	+++	++	+++	+++	+++	+++	+++	+++	+++	+++
	28	++	++	+++	++	+++	+++	+++	+++	+++	++	+++	+++	+++	+++	+++			
Control	7	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+++	+++	+++
	14	+	+	+	+	+++	+++	+++	+++	+++	++	+++	+++	+++	+++	+++	+++	+++	+++
	28	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++			

* Potassium tellurite formed white precipitates at this concentration.

than are nonchromogens. The most interesting finding is that the tuberculin specificity of avian tubercle bacilli could not be differentiated from that of nonchromogens. The animals sensitized with an avian strain reacted to purified proteins of nonchromogens as strongly as to homologous purified protein. Moreover, the purified protein of avian bacilli was capable of producing reactions in the animals sensitized with nonchromogens as great as those of homologous purified proteins.

Susceptibility of unclassified mycobacteria to potassium tellurite: As shown in table 7, the inhibitory dose of potassium tellurite was 0.005 per cent for human strains, 0.01 per cent for bovine strains, 0.05 per cent for avian and unclassified strains, and 1.0 per cent for saprophytic strains. Potassium tellurite is about 500 times more inhibitory for human strains than for saprophytic strains. The other types of mycobacteria are situated between these two extremes.

Discussion

In a previous communication (5), the phage-susceptibility pattern of various mycobacteria was reported in detail. Most of the human, bovine, and murine strains were susceptible to all B-group phages and also to one or two A-group phages, while none of the phages tested were lytic to typical virulent avian strains. Of 55 saprophytes, 38 strains were susceptible to some of the phages. In the present study, all unclassified strains were found to be resistant to any of the phages tested and resembled avian strains in this respect. Since the number of unclassified mycobacteria tested in the present study is limited, further phage-susceptibility studies on many other unclassified strains may disclose that some of them are susceptible to one or more phages. If so, however, the phage-susceptibility test will be useful in classifying the unclassified mycobacteria.

In animal experiments with mycobacteria isolated from dogs, Toda and co-workers (23, 24) have reported that 5 strains with R-type colonies were virulent for guinea pigs, while the remaining 4 strains with S-type colonies were very weakly virulent or avirulent for guinea pigs. From these data, together with cultural characteristics and drug susceptibilities, the former 5 strains have been suspected to be human tubercle bacilli. This might be confirmed by the results obtained in the present study that the phage-susceptibility pattern of the former 5 strains was similar to that of human strains. Although the nature of the latter 4 strains with S-type colonies has not yet been verified, the result of phage-susceptibility tests, together with cultural characteristics and the result of the animal experiments mentioned above, suggest that at least some of these strains would possibly be found to be unclassified mycobacteria on further investigation.

Tuberculin specificity studies of unclassified mycobacteria were first reported by Tarshis and Frisch (6). From the cross-sensitization studies they concluded that an immunologic relationship exists between human and chromogenic strains. The tuberculin employed in their experiment, however, was merely crude culture filtrate except for PPD-S of the human strain. Therefore, their conclusion seems to need further verification. Recently, Affronti (27) reported the results of the cross-immunologic reactions by skin tests, precipitin test, and agar gel technique by use of PPD preparations obtained from unclassified tubercle bacilli. He concluded that the unclassified strains are related to some extent to the human strain and that there was a cross-skin sensitization to all of the PPD preparations. The proteins PPD and PPD-S, however, are composed of three kinds of protein which are different in their potencies, and the quantitative ratio of these proteins contained in PPD and PPD-S is known to vary according to the lot of culture filtrate (8, 28, 29). Tuberculin purified protein preparations containing three kinds of protein, such as PPD and PPD-S, prepared from various mycobacteria, differ in potency and are difficult to standardize for the study of cross-tuberculin reactions. On the other hand, the purified tuberculin protein π used in this experiment is known to contain only one protein component (heated C protein) (25, 26, 30), although its protein content is a little less than PPD-S. Its potency per protein content, therefore, is expected to be uniform irrespective of the strain of mycobacteria from which π was prepared. Accordingly, the usage of π obtained from each test organism will present the most convincing data on the tuberculin specificity. From the present experiment using protein π, it was disclosed that photochromogens are more closely related to human strains than nonchromogens, and nonchromogens are almost the same in their

tuberculin specificity as avian strains. Scotochromogens seem to have an immunologic relationship to nonchromogens.

Recently, PPD-S prepared from a human strain, PPD-B from a nonchromogen, and PPD-Y from a photochromogen were tested by Edwards and Palmer (31) on tuberculous patients and patients infected with unclassified mycobacteria. The sensitivity produced by human strains and by photochromogens could not be differentiated by comparative testing of PPD-S and PPD-Y, although the tests with PPD-S and PPD-B could distinguish the sensitivity produced by human strains and by nonchromogens. These results, indicating that human strains are closely related to photochromogens but not to nonchromogens in tuberculin specificity, coincide with the present data.

Potassium tellurite was reported to be inhibitory for mycobacteria of human, bovine, avian, and saprophytic strains (32–34). The susceptibility of unclassified mycobacteria was found to be similar to that of avian strains and could be differentiated from the other types of mycobacteria in this study.

All present results obtained in phage susceptibility, tuberculin specificity, and potassium tellurite susceptibility indicate that many of the strains of unclassified mycobacteria used in this experiment, especially the nonchromogens, resemble avian strains. The experimental data obtained in this experiment suggest that phage-susceptibility and tuberculin-specificity studies can be used as tools, not only in identifying and classifying the unclassified mycobacteria, but also in clarifying their relation to other types of mycobacteria.

Summary

Ten strains of unclassified mycobacteria were examined for their phage susceptibility, tuberculin specificity, and susceptibility to potassium tellurite.

None of the unclassified mycobacteria used in this experiment were susceptible to any of 10 mycobacteriophages, while many strains of the other types of mycobacteria except avian bacilli were susceptible to one or more mycobacteriophages. Nine strains of mycobacteria isolated from lymph nodes of dogs were also tested for phage susceptibility. Five strains with R-type colonies showed a similar phage-susceptibility pattern to human strains, while the remaining 4 strains with S-type colonies were resistant to all of the phages tested. The unclassified strains showed the same degree of susceptibility to potassium tellurite as the avian strains.

Cross-tuberculin reactions using purified tuberculin protein π revealed that unclassified strains of the photochromogen group and those of the nonchromogen group have their own respective group specificity. The human strain was more closely related to the photochromogens than to nonchromogens in tuberculin specificity. On the other hand, the tuberculin specificity of the nonchromogens was almost identical to that of avian strains. It may be concluded from these facts that nonchromogens are closely related to avian strains of tubercle bacilli.

Sumario

La Susceptibilidad al Bacteriófago y la Especificidad para la Tuberculina de las Micobacterias Inclasificadas. Comunicación Preliminar

Diez cepas de microbacterias inclasificadas fueron estudiadas con respecto a su susceptibilidad al bacteriófago, especificeidad para la tuberculina y susceptibilidad al telurito de potasio.

Ninguna de las micobacterias inclasificadas usadas en este experimento fué susceptible a ninguno de 10 micobacteriófagos, en tanto que muchas cepas de las otras formas de micobacterias, exceptuada la aviaria, fueron susceptibles a uno o más micobacteriófagos. También se comprobaron nueve cepas de micobacterias aisladas de los ganglios linfáticos de perros en cuanto a su susceptibilidad al bacteriófago. Cinco cepas con colonias de forma R revelaron un patrón de susceptibilidad al bacteriófago semejante al de las cepas humanas mientras que las cuatro cepas restantes con colonias de forma S se mostraron resistentes a todos los fagos usados. Las cepas inclasificadas revelaron el mismo índice de sensibilidad al telurito de potasio que las aviarias.

Las reacciones cruzadas a la tuberculina, usando proteína π tuberculínica, revelaron que las cepas inclasificadas del grupo fotocromógeno y las del grupo ancromógeno poseen su propia especificidad respectiva para grupo. La cepa humana se mostró más afín de las fotocromógenas que de las ancromógenas en su especificidad para la tuberculina. Por otro lado, la especificidad para la tuberculina de las ancromógenas fué casi idéntica a la de las cepas aviarias. Cabe deducir de estos datos que las cepas ancromógenas están intimamente relacionadas con las aviarias.

Resume

Sensibilité au bactériophage et spécificité tuberculinique de mycobactéries non classifiées. Rapport préliminaire

Dix souches de mycobactéries non classifiées furent examinées sous l'angle de leur sensibilité aux bactériophages, leur spécificité tuberculinique et leur sensibilité à la tellurite de potassium.

Aucune des mycobactéries non classifiées utilisées dans cette expérience ne fut sensible à aucun des 10 mycobactériophages tandis que plusieurs souches d'autres types de mycobactéries, excepté le type aviaire, furent sensibles à un ou plusieurs mycobactériophages. Neuf souches de mycobactéries isolées de ganglions lymphatiques de chiens furent aussi étudiées pour leur sensibilité aux bactériophages. Cinq souches ayant des colonies de type R montrèrent un genre de sensibilité semblable à celle des souches humaines tandis que les quatre autres souches, de type S, furent résistantes vis à vis de tous les bactériophages de l'expérience. Des souches non classifiées montrèrent le même degré de sensibilité que les souches aviaires vis à vis de la tellurite de potassium.

Des contre-réactions utilisant les protides de tuberculine purifiée π montrèrent que les souches non classifiées du groupe photochromogène et celles du groupe non chromogène ont leur propre sensibilité respective. La souche humaine se rapproche davantage du groupe photochromogène que du groupe non chromogène dans sa sensibilité tuberculinique. D'un autre coté, la sensibilité tuberculinique du groupe non chromogène fut presqu'identique à celle des souches aviaires. On peut conclure à partir de ces faits que le groupe non chromogène est proche des souches aviaires.

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Addendum

Further studies on the phage susceptibility of unclassified mycobacteria (Takeya and Mori, unpublished data) have disclosed that several strains were susceptible to C-3 and/or A-6 phages when the organisms grown on glycerol agar medium, instead of Dubos Tween-albumin medium, were used as the inoculum to the "spot test." Moreover, many unclassified strains were found susceptible to phages when these organisms were tested on glycerol agar medium instead of on Ogawa egg medium.

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PLEUROPULMONARY AMEBIASIS

A Review with an Analysis of Ten Cases

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Introduction

Although pleuropulmonary amebiasis is considered to be a rare disease, an ever increasing number of cases are reported as clinicians become familiar with this syndrome. Although hepatic involvement is recognized as the most frequent complication of amebiasis, pulmonary involvement ranks next in frequency.

The exact incidence of pulmonary involvement in amebiasis is unknown. Radke (1) reports one case of pulmonary involvement in 101 cases of amebiasis. Among 388 cases of amebic dysentery, Ochsner and DeBakey in 1935 (2) reported 7 cases (13.5 per cent) complicated by extension of the suppurative process to the thorax, with involvement of the lungs in 6 cases and the pleura in one. In 1936, Ochsner and DeBakey (3) reported that 15.7 per cent of their 95 cases of amebic hepatic abscess had pleuropulmonary complications. In this same series, males predominated in the ratio of 15 to 1. Webster in 1956 (4) reported 6 cases of pleural or pneumonic involvement representing an incidence of 6.3 per cent of 95 cases of amebiasis.

Pulmonary and hepatic involvement are believed to be secondary to intestinal infestation in all cases. Pulmonary amebiasis is usually secondary to hepatic involvement. There may be contiguous spread through the diaphragm, transdiaphragmatic lymphatic extension, or embolic spread through the circulation (5). About 75 per cent of cases occur by a rupture of a liver abscess directly through the diaphragm (6).

Cases of the primary form of pulmonary amebiasis caused by amebae reaching the respiratory tract by direct embolism from the intestinal tract are rare, but such cases are on record (7). Such cases, in which there was no history of dysentery, hepatic involvement, or *Endamoeba histolytica* in the feces, have been reported by Chaudhuri and Chaudhuri (8) and by Webster (4).

Ochsner and DeBakey (3) analyzed 153 cases collected from the literature. They divided the cases according to their own classification as follows: 14.3 per cent had hematogenous pulmonary abscess without liver involvement; 10.4 per cent had hematogenous pulmonary abscess and independent liver disease; 37.2 per cent had pulmonary abscess extending from a liver abscess; 19.6 per cent had bronchopulmonary fistulas with little pulmonary involvement; and 17.6 per cent had empyema extending from a liver abscess.

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Clinical Material

The present series consists of 10 cases of pleuropulmonary amebiasis observed in a series of 249 cases of amebiasis. *Endamoeba histolytica* was identified in the 249 cases by different technicians, but in each case the microscopic findings were reviewed and confirmed by the writer. There were 48 cases of recognizable hepatic amebiasis. None of this series of 10 patients had been military veterans of foreign service. In fact, none of the 10 had ever been outside the continental United States. Only 4 of the patients had had frank dysentery. All 10 patients were white, and 6 of the 10 were females. Only one person lived in a rural area. The ages of the patients ranged from thirty-six to eighty-two years.

Symptomatology and Diagnosis

The symptoms of pleuropulmonary amebiasis are variable (table 1) because the physical findings are very different from case to case. The symptoms may be those of a lung abscess, consolidation, pleurisy, pleural effusion, empyema, or bronchobiliary fistula. Cough is the most frequent complaint. Expectoration of a copious "chocolate sauce" pus is indicative of a communication between a liver abscess and a bronchus (3). This type of sputum is generally accepted as virtually diagnostic of pulmonary amebiasis. The sputum may be yellow or creamy when bacterial infection coexists. Pain in the right side of the chest and low-grade fever are

TABLE 1

RESPIRATORY SYMPTOMS IN TEN PATIENTS WITH PLEUROPULMONARY AMEBIASIS

Symptoms	Number of Patients
Cough	10
Fever	10
Cardiac arrhythmia	8
Pain in right side	8
Wheezing	8
"Apple butter" sputum	7
Hemoptysis	6
Hoarseness	6
Chills	6
Gagging	5
Strong-smelling sputum	3
Greenish-yellow sputum	3
Strangling	1
Soreness of right ribs	1

usually encountered. Cachexia due to the chronic illness may be present. As would be expected, there may be a history of present or past diarrhea associated with tenesmus, diarrhea alternating with constipation, or very chronic constipation (table 2).

The main physical findings may be those of dullness to percussion, inspiratory lag on the affected side, and rales in the involved area (table 3). In the case of amebic bronchitis, there may be sibilant or sonorous rales which vary and may disappear on coughing. In the majority of cases there may be hepatomegaly and tenderness along the right costal margin. In many cases the clinical and roentgenographic findings do not reflect the gravity of the disease. The temperature is a variable factor and may be a septic type with chills, or there may be a very slight elevation in the afternoon. At times there is no demonstrable elevation of the temperature.

The amebic lesions cannot be differentiated from other diseases of the chest solely by roentgenography (table 4), but fluoroscopic examination of the diaphragm is frequently very helpful. The presence of a triangular pneumonic shadow with or without a lung abscess superior to the right diaphragm is often found. Ochsner and DeBakey (3) state that the shadow is characteristically triangular-shaped, with the base below and the apex extending toward the hilum above. Alarcón (9) suggests that the roentgenographic findings of a crescent-shaped shadow less dense and smaller than that of the liver silhouette superimposed above the liver is pathognomonic. Cavitation of the lungs is not uncommon.

The only laboratory finding which is definitive is the demonstration of *Endamoeba histolytica* in the sputum or aspirated material. This exami-

TABLE 2

SYSTEMIC SYMPTOMS IN TEN PATIENTS WITH PLEUROPULMONARY AMEBIASIS

Symptoms	Number of Patients
Flatulence	10
Right upper quadrant pain	9
Tenesmus	8
Eructation	8
Vague indigestion	8
Weight loss	7
Fatigue	7
Vertigo	7
Nausea	6
Mucus in stools	6
Nasal allergy	5
Diarrhea	5
Headache	5
Tinnitus	5
Perspiration	5
Arthralgia	4
Myalgia	4
Frank dysentery	4
Anorexia	4
Intermittent diarrhea	3
Blood in stool	3
Backache	1
Constipation	1

TABLE 3

PHYSICAL SIGNS IN TEN PATIENTS WITH PLEUROPULMONARY AMEBIASIS

Signs	Number of Patients
Palpable liver	9
Right upper quadrant tenderness	8
Dullness of right lower lobe	8
Moist rales, right base	8
Rhonchi, right hilar area	8
Decreased fremitus, right lung base	7
Respiratory lag, right lung base	7
Decreased breath sounds, right lung base	6
Cardiac arrhythmias	5
Nasopharyngitis	5
Pleuritic rub, right lower lobe	4
Moist rales, right lung hilus	3
Bilateral hilar rales	3
Rectosigmoid ulceration	3
Right lower quadrant tenderness	3
Proctitis	1

TABLE 4

ROENTGENOGRAPHIC FINDINGS IN TEN PATIENTS WITH PLEUROPULMONARY AMEBIASIS

Findings	Number of Patients
Elevation and fixation of right diaphragm	7
Infiltration of right pulmonary base	6
Increase of right hilar shadow	6
Infiltration of right lower lobe	5
Increased bronchovascular markings of right pulmonic base	4
Elliptical infiltration in right pulmonic base	4
Bilateral bronchovascular infiltration	3
Unresolved pneumonia	3
Pleurisy overlying right diaphragm	3
Appearance of bronchiectasis of right pulmonic base	1

nation is said to be negative in 20 to 40 per cent of proved cases (9). Chakravarti (10) recommends that the sputum be searched for amebae immediately after expectoration, and that the specimen be mounted in normal saline. The blood counts are not characteristic. There is usually a mild secondary anemia. A moderate leukocytosis of 9 to 12 thousand is the rule, but this may be higher in bacterial infection. There may not be a corresponding rise in the polymorphonuclear leukocytes. Eosinophilia is not present. A positive complement-fixation test is strong circumstantial evidence for the diagnosis (6), but a negative complement-fixation test does not exclude the presence of the disease. A positive stool examination may be used as suggestive evidence of pulmonary amebiasis when symptoms are present. Finally, the diagnosis of pleuropulmonary amebiasis by a therapeutic trial on emetine hydrochloride is an accepted technique (11). Emetine is a specific in the treatment of amebiasis and is accepted as the valid test of the etiology of pulmonary amebiasis (6). The specific action of emetine is described by Rogers (12). Chloroquine diphosphate is noted by Sullivan and Bailey (6) as less toxic and also as an effective diagnostic agent.

DIFFERENTIAL DIAGNOSIS

The diagnosis of pleuropulmonary amebiasis (table 3) should always be considered when there are unexplained abnormalties at the base of the right lung, particularly when accompanied by low-grade fever. The most important point in the diagnosis is to keep the disease entity in mind. Such cases are often diagnosed and treated for long intervals as pleurisy, pleural effusion, or lung abscess, without consideration of amebiasis. The disease may be confused with bronchopneumonia, bronchiectasis, or basilar tuberculosis. Deb (13) mentions the frequency of pulmonary amebiasis in tea-garden practice in India and mentions the frequent confusion with tuberculosis. The roentgenographic findings of right basilar tuberculosis not substantiated by the finding of acid-fast tubercle bacilli should arouse suspicion of amebiasis. The different pyogenic infections of the lungs—typhoid fever, paratyphoid fever, brucellosis, and malaria—can easily be confused in the differential diagnosis.

A subphrenic abscess may be difficult to differentiate because of the elevation of the right diaphragm. Pulmonary infarction in the right base may be diagnosed by early bloody sputum, very sudden onset, and by recent normal pulmonary findings.

PROGNOSIS AND THERAPY

The prognosis of pleuropulmonary amebiasis depends largely upon the early institution of adequate specific therapy, as previously stated. A therapeutic trial with emetine or chloroquine diphosphate will produce a satisfactory response if amebiasis is present and amebae are not found. An amebic abscess usually responds rapidly to these drugs. Applebaum and Frankel (14) recommend a therapeutic trial with emetine in any obscure abscess of the lung when amebiasis is suspected.

Quinacrine is mentioned by Radke (15) as a safe and effective agent in the treatment of amebiasis. It is said to reach the pleural and hepatic lesions in effective concentrations when 0.1 gm. is administered four times daily for eighteen days. The drug of choice according to Sodeman and Lewis (16) is chloroquine diphosphate. This drug is readily absorbed and may be concentrated in the liver and thoracic organs up to 500 times its concentration in the plasma. The destruction of the extra-intestinal parasites has been found to be effective. Chloroquine is much less toxic than emetine. The dosage may be scheduled as 1 gm. daily for two days, then 0.5 gm. daily for two or three weeks. If chloroquine diphosphate gives unsatisfactory results, then emetine hydrochloride should be administered by deep subcutaneous injection.

Emetine is effective against the trophozoites, but is ineffective against amebic cysts. It is contraindicated in myocardial disease, marked renal impairment, and marked hypertension. Therefore, a painstaking evaluation of the cardiovascular system should precede the usage of the drug. Toxic symptoms may include acute fall in blood pressure, cardiac irregularities, weakness and pain, or even paralysis of the skeletal muscles, nausea, vomiting, sudden appearance of dyspnea, and prostration. Myocardial degeneration may occur. Inversion or depression of the T-wave in the electrocardiogram should indicate a serious side effect. The dosage of emetine should be 0.5 grain (0.032 gm.) twice daily, or 1.0 grain (0.065 gm.) daily for six to ten days. The clinical response to emetine or chloroquine is dramatic, usually in four to six days, and recovery may be evident in two to three weeks. The aggravation of the symptoms of amebiasis by emetine is often experienced during the first few injections.

The appropriate administration of other antimicrobial drugs may be necessary in the presence of bacterial infection. A nutritious diet, high in proteins, carbohydrates, and vitamins, is a helpful adjunct in the treatment. Bed rest is essential during treatment with emetine. The successful treatment of hepatic abscess in several patients is mentioned by Klatskin (17). The criteria for cure that he lists are complete absence of pain and fever, absence of liver enlargement, disappearance of subcostal tenderness, and normal leukocyte count and sedimentation rate.

Shaw (18) lists the following indications for surgery of the pleuropulmonary complications of amebiasis when: (*1*) bacterial infection has occurred, (*2*) empyema does not respond to repeated aspirations, (*3*) a persistent abnormal channel exists between the bile duct system and the bronchi, and (*4*) fibrosis of pulmonary tissue is present as a result of an abscess with continued symptoms. When surgery is necessary, formal thoracotomy with complete removal of the diseased tissue is preferable to drainage procedures.

Findings in the Present Series of Ten Cases

Endamoeba histolytica with the typical morphologically accepted characteristics has been demonstrated in the sputum of the patients of 10 cases herein reported. *Endamoeba histolytica* cysts were found exclusively in one case. Bile was present in the sputum of 8 of the patients, indicating that a bronchobiliary communication either was or had been present. The sputum varied from yellow-greenish mucoid to dark brown "apple butter" sputum. In the sputum specimens which were yellow or yellow-green, more bacterial infection seemed to exist. The sputum was examined immediately, while warm, and was suspended in saline in all of the cases. All sputum specimens were negative for tubercle bacilli by microscopy and culture, and the tuberculin reaction was negative in all of the cases.

The most frequent symptom observed was cough (*see* table 1). At the onset, it was dry or frequently produced mucoid sputum which was very scant. In the later stages, the typical "chocolate sauce," "strawberry jelly," or "apple butter" type indicative of a bronchobiliary fistula was observed.

Pain in the right lower chest, either anterior or posterior, was noted in 8 of the cases. The pain usually radiated to the shoulder and was very persistent. Low-grade fever of a septic type was frequently observed. Right upper quadrant pain, hepatomegaly, and varying degrees of impairment of liver function were present in 9 of the cases.

Total leukocyte counts ranging from 9,700 to 22,500 were present. A moderate neutrophilia was observed in 6 cases. Eosinophilia was not present in any of the 10 patients in the series. The erythrocyte sedimentation rates ranged from 17 to 48 mm. at the height of the infections, but returned to normal as the disease subsided. A mild anemia was noted in 5 patients, and moderately severe anemia in one case. A positive amebic complement-fixation test was observed in 4 patients, and doubtfully positive reactions were observed in 5 other patients during the peak of the illness.

The stools were positive for *Endamoeba histolytica* in all 10 cases of the present series. When amebiasis is suspected, the continued search for the organisms in the stool specimens is imperative. When patients are ambulatory and have diarrhea, they are encouraged to come to the laboratory for evacuation so that an immediate search may be initiated. If the patient is hospitalized, the stool is sent to the parasitologist for immediate attention. If the patient is

not diarrheal, then a sample is obtained following a saline purge. All specimens are examined by direct search, flotation methods, and MIF stain.

The roentgenographic findings in the 10 patients are presented in table 4. Elevation and some degree of fixation of the right diaphragm was noted in 7 cases. Elliptical infiltrations in the right base which resembled atypical pneumonias were noted in 4 instances, and roentgenographic evidence of an unresolved pneumonia was present in 3 patients. Infiltration that resembled a bronchiectasis in the right pulmonary base was found in one case. An infiltration in the right lower lobe and enlarged hilar lymph nodes bilaterally were recognized in 6 cases.

A pleuritic rub over the right lower lobe was audible early in 3 cases, evidence of acute fibrinous pleurisy. One of the patients experienced the onset of amebic hepatitis and the subsequent development of an amebic bronchobiliary fistula, which were evidenced by the brownish ("apple butter") bile-stained amebic-bearing sputum the patient produced while receiving chemotherapy for intestinal amebiasis.

General Comment

The anatomic site of the amebic infection and the nature of the pathologic process should determine the type of drug used. Certain drugs are effective against the organisms in the intestinal tract whereas others attack the protozoa which have invaded the tissues. Some drugs possess both types of action. It is conceivable that there may be some degree of extra-intestinal amebic invasion in a large percentage of amebic infections. Therefore, it seems reasonable to employ a therapeutic regimen with drugs of different types of action. Such a combination, composed of glycobiarsol (Milibis®) with chloroquine (Aralen®) phosphate, has been very effective in the present series, and no recurrences have thus far been observed. The treatment has consisted of chloroquine alone or in combination with other drugs in 6 cases. Diiodohydroxyquinoline glycolylarsanilate (Triamar®) was successfully used in 3 cases. Emetine hydrochloride was employed in only one case. A drug to combat the intestinal organisms was thought to be necessary in each case. Other antimicrobials were employed when an associated bacterial infection was thought to be present.

The criteria for cure in these cases have been: (*1*) the return of the clinical pulmonary, hepatic, and intestinal symptoms and findings to normal; (*2*) the disappearance of the roentgenographic signs; (*3*) the return of the sputum to normal; (*4*) normal stools; and (*5*) disappearance of leukocytosis, elevation of the erythrocyte sedimentation rate, and fever. In each case, the general appearance of the patient has improved from that of a chronically debilitated person to normal. Finally, let it be said that pleuropulmonary amebiasis should be suspected in all bizarre disease in the lower right chest whether or not amebae have been demonstrated in the stool. The absence of recognizable hepatic disease should not necessarily preclude the diagnosis. The diagnosis of pleuropneumonic amebiasis depends in large measure upon awareness that such a condition exists.

Summary

Ten cases of pleuropulmonary amebiasis, or 4.02 per cent among 249 patients with intestinal amebiasis, are reported. Nine of the cases occurred among 48 cases of recognizable hepatic amebiasis, and one appeared as an amebic bronchitis without demonstrable liver involvement. The diagnoses in the 10 patients were substantiated by the demonstration of *Endamoeba histolytica* in the sputum in the form of trophozoites in 9 cases, and as cysts in one case. A bronchobiliary communication existed in 8 of the patients. Six patients were sucessfully treated with chloroquine diphosphate, 3 with diiodohydroxyquinoline glycolylarsanilate (Triamar®), and one with emetine hydrochloride. Surgical intervention was not necessary in any patients in this series.

Sumario

Amibiasis Pleuropulmonar. Reseña con Análisis de Diez Casos

Se presentan 10 casos de amibiasis pleuropulmonar, o sea 4.02 por ciento, entre 249 enfermos que tenían amibiasis intestinal. Nueve de ellos ocurrieron en 48 casos de reconocible amibiasis hepática y uno en forma de bronquitis amibiana sin signos de invasión hepática. El diagnóstico en los 10 enfermos quedó corroborado por el descubrimiento de trofozoítos de la *Endamoeba histolytica* en el esputo en 9 y de quistes en uno. En 8 de los enfermos existía una comunicación broncobiliar. A 6 enfermos se les trató con éxito con bifosfato de cloroquina, a 3 con glicolilarsanilato de diyo-

hidroxiquinolina (Triamar®) y a uno con clorhidrato de emetina. No resultó necesaria la intervención cruenta en ningún enfermo de esta serie.

Resume

Amibiase pleuropulmonaire. Revue avec analyse de dix cas

L'auteur rapporte dix cas d'amibiase pleuropulmonaire, soit 4,02% parmi 249 malades ayant une amibiase intestinale. Neuf cas se présentèrent parmi 48 cas d'amibiase hépatique avérée et un cas ayant une bronchite amibienne ne fournit aucune preuve d'atteinte hépatique. Le diagnostique chez les dix malades fut vérifié par l'évidence de formes trophozoites d'*Entamoeba histolytica* dans l'expectoration de 9 malades, et par l'évidence de kystes chez un autre malade. Une communication bronchobiliaire fut trouvée chez 8 malades. Six malades furent soignés avec succès par le sulfate de chloroquine, 3 par le glycolylarsanilate de diiodohydroxyquinoline (Triamar®) et un par l'hydrochloride d'émétine. Aucune intervention chirurgicale ne fut nécessaire dans ce groupe de malades.

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SCREENING PROCEDURES FOR PULMONARY DISEASE IN PRENATAL PATIENTS[1, 2, 3]

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INTRODUCTION

Chest roentgenograms have been part of the routine prenatal studies at the Hospital of the Woman's Medical College of Pennsylvania for years. Early in 1957, because of adverse publicity about radiation hazards, the Department of Preventive Medicine suggested limiting chest roentgenograms to tuberculin reactors. The Obstetrical Department was unwilling to do this because important nontuberculous disease had not infrequently been discovered by roentgenography. To determine the volume of significant disease which would be missed were roentgenograms limited to reactors, a pilot project of concurrent tuberculin tests and photofluorograms was established.

METHOD

During the seven-month period, June 1 through December 31, 1957, of 310 prenatal clinic patients at Woman's College Hospital, 304 (table 1) had both tuberculin tests and roentgenography. The first-strength PPD (0.00002 mg.) was used, followed by the intermediate dose (0.0001 mg.) for nonreactors. A reactor was defined as an individual in whom an area of induration 5 mm. or larger was measured at the injection site forty-eight to seventy-two hours after the test.

An analysis was made of tuberculin reactors, reaction size, roentgenographic appearance, and of patient cooperation in returning for tuberculin test readings.

Failure to return: After 150 tests, it was noted that about half of the patients had failed to return for test readings. Therefore, a physician and a medical student were assigned to patient education. This effort would not be feasible for a routine program. There were many good reasons for failure to return: lack of carfare, difficulty in finding a "sitter" for other children, et cetera.

Change in methodology: Because of these administrative difficulties, the study was expanded somewhat. Tuberculin tests and chest roentgenograms were done also on all obstetrical ward patients at Woman's College Hospital from January 1 through May 31 and from September 1 through December 31, 1958. To compare the situation in a large city hospital, a similar program for obstetrical admissions was conducted at Philadelphia General Hospital from June 16 through September 1, 1958. However, at Philadelphia General Hospital, 30 per cent of the patients had been discharged within forty-eight hours after the first-strength tuberculin had been given, so the first test could not be read. The remaining patients were discharged before an intermediate test could have been read, so it was not given.

This experience is somewhat analogous to that of Custer (1) who, reporting on a tuberculin testing study at a general hospital in South Bend, Indiana, states that 454 of almost 2,500 patients tested required field nurses to complete readings after patients had left the hospital.

A group of private obstetrical patients at Woman's College Hospital were tested over the same period as the obstetrical ward patients.

RESULTS

Woman's College Hospital: Prenatal Clinic and Ward Patients

Twenty-two per cent of the patients from the prenatal clinic and wards reacted to the first-strength PPD and 10 per cent to the intermediate strength (figure 1). There is an impression that reactivity to tuberculin is depressed during pregnancy. Paraf and Desbordes (2) reported in 1951 that about 15 to 20 per cent of women in the last trimester of pregnancy or post partum have anergy to tuberculin. Lurie (3) has done some experimental work in this field. He and his associates found that tuberculin reactivity in rabbits is depressed during estrogen administra-

[1] From the Woman's Medical College of Pennsylvania, Philadelphia General Hospital, and the Philadelphia Department of Health, Philadelphia, Pennsylvania.

[2] This study was assisted in part by three medical student fellowships from the United States Public Health Service.

[3] Presented before the Medical Session, as part of *Section 2C*, at the annual meeting of the American Trudeau Society, Chicago, Illinois, May 25, 1959.

TABLE 1

COMPOSITION OF THE GROUP OF PRENATAL CLINIC PATIENTS*

Hospital of the Women's Medical College of Pennsylvania

Age	Nonwhite		White		Total	
	Number	Per Cent of 304	Number	Per Cent of 304	Number	Per Cent of 304
years						
15–19	29	10	10	3	39	13
20–24	82	27	18	6	100	33
25–29	72	24	7	†	79	26
30–34	49	16	6	†	55	18
35–39	21	7	3	†	24	8
40–44	6	†	1	†	7	†
Totals	259	86	45	14	304	100

* Basis: 304 patients who had both PPD tests and photofluorograms.

† Base too small for statistical significance.

tion. The increase of estrogen during pregnancy may be the mechanism by which tuberculin reactivity is depressed.

The total reactor rate of 28 per cent in the present study (figure 1) is based on 60 reactors to first-strength and 23 reactors to intermediate-strength PPD among 285 clinic patients (table 2) plus 47 reactors to first-strength and 11 reactors to intermediate-strength PPD among 211 ward patients (table 3). This rate may be lower than the figure would have been in these same women had they been tested before pregnancy.

Wood and associates (4) stress the enhanced clinical significance of large tuberculin reactions. Twenty-eight of the 107 reactors to first-strength PPD had areas of induration larger than 20 mm. (table 4).

No significant roentgenographic abnormality of the chest was found among the 524 patients, whether or not they were reactors. The one recent case of active pulmonary tuberculosis on the obstetrical service at this hospital was an unregistered mother who came to the emergency ward in active labor and was delivered immediately. Her sputum contained many tubercle bacilli when it was examined two days post partum because of fever and an abnormal chest roentgenogram.

Woman's College Hospital: Private Obstetrical Patients

All but one of the tuberculin tests on 153 private obstetrical patients at the Woman's College Hospital were read. The total reactor rate of 15 per cent was about half the figure for ward and clinic patients regardless of age (table 5). No reaction was larger than 20 mm.

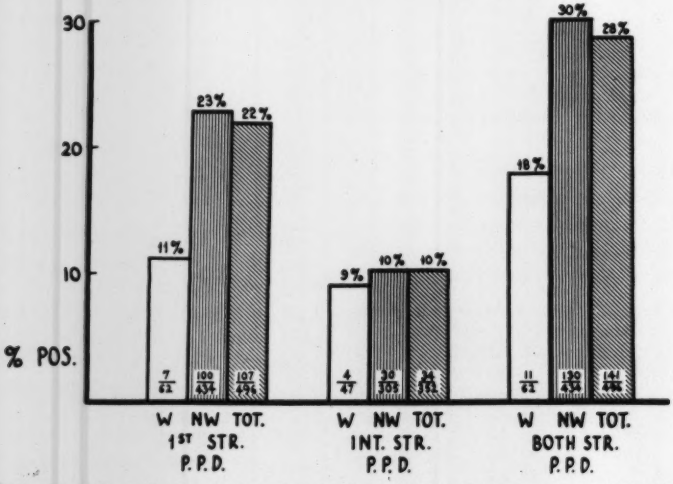

FIG. 1. Reactors by tuberculin strength and race of the prenatal clinic and obstetrical ward patients of the Woman's College Hospital.

TABLE 2

REACTORS BY TUBERCULIN STRENGTH, AGE, AND RACE OF PRENATAL CLINIC PATIENTS*
Hospital of the Woman's Medical College of Pennsylvania

	Nonwhite			White			Total		
	Less than 25 Years	More than 25 Years	Total	Less than 25 Years	More than 25 Years	Total	Less than 25 Years	More than 25 Years	Total
First-strength PPD given	111	148	259	28	17	45	139	165	304
Not read	7	6	13	5	1	6	12	7	19
Read	104	142	246	23	16	39	127	158	285
Nonreactors	90	100	190	21	14	35	111	114	225
Reactors	14	42	56	2	2	4	16	44	60
Intermediate-strength PPD not given	2	1	3	—	—	—	2	1	3
Intermediate-strength PPD given	88	99	187	21	14	35	109	113	222
Not read	6	2	8	2	2	4	8	4	12
Read	82	97	179	19	12	31	101	109	210
Nonreactors	74	85	159	18	10	28	92	95	187
Reactors	8	12	20	1	2	3	9	14	23
Total reactors to first- and intermediate-strength PPD	22	54	76	3	4	7	25	58	83

* Basis: 304 patients who had both PPD tests and photofluorograms.

TABLE 3

REACTORS BY TUBERCULIN STRENGTH, AGE, AND RACE OF OBSTETRICAL WARD PATIENTS*
Hospital of the Woman's Medical College of Pennsylvania

	Nonwhite			White			Total		
	Less than 25 Years	More than 25 Years	Total	Less than 25 Years	More than 25 Years	Total	Less than 25 Years	More than 25 Years	Total
First-strength PPD given	90	104	194	12	14	26	102	118	220
Not read	4	2	6	1	2	3	5	4	9
Read	86	102	188	11	12	23	97	114	211
Nonreactors	76	68	144	11	9	20	87	77	164
Reactors	10	34	44	—	3	3	10	37	47
Intermediate-strength PPD not given	2	1	3	—	—	—	2	1	3
Intermediate-strength PPD given	74	67	141	11	9	20	85	76	161
Not read	5	10	15	2	2	4	7	12	19
Read	69	57	126	9	7	16	78	64	142
Nonreactors	68	48	116	8	7	15	76	55	131
Reactors	1	9	10	1	—	1	2	9	11
Total reactors to first- and intermediate-strength PPD	11	43	54	1	3	4	12	46	58

* Basis: 220 patients who had both PPD tests and photofluorograms.

Philadelphia General Hospital: Obstetrical Patients

Of the 621 obstetrical patients tested at the Philadelphia General Hospital, 189 left before their tests could be read (table 6). Only 23 of the 40 white patients had their tests read, so the analysis is based almost entirely on 409 Negro women whose tests were read. Eighteen per cent (38 of 214) of the women less than twenty-

five years old reacted to first-strength PPD (table 6) compared with 12 per cent of the same age group at Woman's College Hospital (figure 2). Reactor rates were the same in both hospitals for women older than twenty-five: 29 per cent at Philadelphia General Hospital (table 6) and 30 per cent at Woman's College Hospital (figure 2). Sixteen per cent of the reactions of patients at Philadelphia General Hospital were larger than 20 mm. while 26 per cent of the patients at Woman's College Hospital had reactions of more than 20 mm. (table 4).

One case of proved sarcoidosis was detected roentgenographically in a nonreactor.

Discussion

It is desirable to avoid all unnecessary radiation, especially in pregnant women. This study suggests that tuberculin testing as a substitute

TABLE 4

Size of Reaction to First-Strength PPD by Age and Race: 524 Obstetrical Ward and Prenatal Clinic Patients

Hospital of the Woman's Medical College of Pennsylvania

	Nonwhite						White						Total					
	Less than 25 Years		More than 25 Years		Total		Less than 25 Years		More than 25 Years		Total		Less than 25 Years		More than 25 Years		Total	
	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Given	201		252		453		40		31		71		241		283		524	
Read	190		244		434		34		28		62		224		272		496	
Total reactors	24	100	76	100	100	100	2	100	5	100	7	100	26	100	81	100	107	100
Induration less than 10 mm.	4	17	13	17	17	17	1	*	2	*	3	*	5	19	15	18	20	19
Induration 10–19 mm.	14	58	43	57	57	57	1	*	1	*	2	*	15	58	44	54	59	55
Induration more than 20 mm.	6	25	20	26	26	26	—	—	2	*	2	*	6	23	22	28	28	26

* Base too small for statistical significance.

TABLE 5

Size of Reaction to First-Strength PPD by Age and Race of 153 Private Obstetrical Patients

Hospital of the Woman's Medical College of Pennsylvania

	Nonwhite						White						Total					
	Less than 25 Years		More than 25 Years		Total		Less than 25 Years		More than 25 Years		Total		Less than 25 Years		More than 25 Years		Total	
	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Given	6		12		18		38		97		135		44		109		153	
Read	6		12		18		38		96		134		44		108		152	
Total reactors	1	100	1	100	2	100	2	100	6	100	8	100	3	100	7	100	10	100
Induration less than 10 mm.	—	—	—	—	—	—	1	*	4	*	5	*	1	*	4	*	5	*
Induration 10–19 mm.	1	*	1	*	2	*	1	*	2	*	3	*	2	*	3	*	5	*
Induration more than 20 mm.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

* Base too small for statistical significance.

TABLE 6

SIZE OF REACTION TO FIRST-STRENGTH PPD BY AGE AND RACE OF 621 OBSTETRICAL WARD PATIENTS

Philadelphia General Hospital

	Nonwhite						White						Total					
	Less than 25 Years		More than 25 Years		Total		Less than 25 Years		More than 25 Years		Total		Less than 25 Years		More than 25 Years		Total	
	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Given	305		276		581		18		22		40		323		298		621	
Read	206		203		409		8		15		23		214		218		432	
Total reactors	37	100	59	100	96	100	1	100	2	100	3	100	38	100	61	100	99	100
Induration less than 10 mm.	12	32	15	25	27	28	—	—	—	—	—	—	12	37	15	25	27	27
Induration 10–19 mm.	19	51	35	63	54	56	1	*	1	*	2	*	20	53	36	59	56	56
Induration more than 20 mm.	6	16	9	12	15	16	—	—	1	*	1	*	6	15	10	16	16	16

* Base too small for statistical significance.

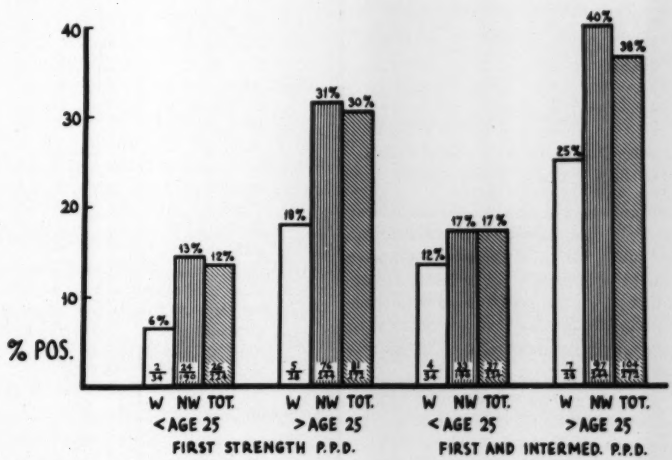

FIG. 2. Reactors by tuberculin strength, age, and race of the prenatal clinic and obstetrical ward patients of the Woman's College Hospital.

for prenatal chest roentgenograms is impracticable for clinic patients and obstetrical patients in large city hospitals. Each community must evaluate its own situation. If administrative problems make routine tuberculin testing difficult and a significant percentage of patients react to tuberculin, a properly monitored chest roentgenographic program appears justifiable. This is especially true in large city hospitals like Philadelphia General Hospital where 33 per cent of obstetrical patients arrive in active labor, never having attended the prenatal clinic. The segment of unregistered mothers is likely to furnish unsuspected tuberculosis.

On the other hand, if reactor rates are less than 10 or 15 per cent as for the private obstetrical patients at Woman's College Hospital, a tuberculin testing program appears advisable in order

to avoid unnecessary exposure of nonreactors to X-ray. Provision should be made for home visits by qualified nurses in those instances in which patients fail to return for readings.

Chest roentgenograms have the advantage of alerting the staff at a time when appropriate diagnostic studies can be carried out promptly. By contrast, if the first screening consists of post-partum tuberculin testing, even if the reading is completed and the roentgenograms taken on reactors to tuberculin before hospital discharge, there may be inadequate time for definitive studies before the patient leaves the hospital.

Summary

The decision whether or not tuberculin testing may wisely be substituted for routine chest roentgenograms should be based on the proportion of tuberculin tests read, the proportion of reactors among the prenatal patients, and the proportion of significant intrathoracic disease among nonreactors.

Sumario

Los Procedimientos de Tria para Enfermedad Pulmonar en los Casos Prenatales

La decisión en cuanto a si puede o no emplearse acertadamente la comprobación con tuberculina en vez de las radiografías torácicas corrientes debe basarse en la proporción de pruebas tuberculínicas estudiadas, la proporción de reactores entre las enfermas prenatales y la proporción de importante enfermedad intratorácica entre las que no reaccionan.

Resume

Méthodes prénatales "passant au crible" les parturientes pour détecter les maladies pulmonaires

Pour décider s'il est sage de substituer à la réaction à la tuberculine des examens radiographiques systématiques, il faudrait se baser sur la proportion de réactions à la tuberculine lues, la proportion de sujets réagissant parmi les femmes enceintes et la proportion de maladies intrathoraciques sérieuses trouvée parmi les sujets ne réagissant pas.

Acknowledgment

The writers wish to express their deep gratitude to Dr. Marie Capitanio and Lenora Gray of Woman's College Hospital, and to Bernard Broad of Temple University, who did most of the actual tuberculin testing; to Mrs. Elizabeth Fleisher for her editorial assistance, and to Mrs. Betty MacKinlay for secretarial help in the preparation of this manuscript.

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COMPARISON OF GEL PRECIPITIN AND SENSITIZED ERYTHROCYTE TECHNIQUES FOR THE DETECTION OF ANTIBODY IN THE SERUM OF TUBERCULOUS AND NONTUBERCULOUS PATIENTS[1, 2]

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INTRODUCTION

Clinical evaluations of the hemagglutination and hemolytic tests for the detection of a specific antibody response in tuberculous subjects have most frequently been made with Old Tuberculin (OT) as the erythrocyte-sensitizing antigen (1–3). The lack of specificity demonstrated in these studies has generally been attributed to the crude nature of the antigen. Unfortunately, no practicable means has been found for isolating specific antigens from OT or unheated culture filtrates although experimental studies have revealed their existence in these substances (4, 5).

In view of the known complexity of the heated and unheated culture filtrates of the mycobacteria, it was of great interest when Parlett and his associates (6) found that serum from 48 of 53 tuberculous patients gave positive precipitin reactions in gel while only 2 of 38 specimens from clinically well persons were positive when tested with unheated concentrated culture filtrates of the virulent H37Rv strain of tubercle bacilli. In contrast, Burrell and co-workers (7) were able to detect precipitin lines in agar plates, using OT as antigen, in only 5 of 40 serum specimens from tuberculous patients. Thirty-two of these patients reacted with OT-sensitized erythrocytes in hemolytic or hemagglutination tests.

The present investigation was undertaken to obtain additional comparative data on the reactivity of serum from tuberculous and nontuberculous patients when tested by the gel precipitin method used by Parlett and associates (6) and by the hemagglutination and hemolytic tests. The latter techniques are referred to collectively in this paper as the sensitized erythrocyte tests.

[1] From the Trudeau Laboratory of the Trudeau Foundation, Inc., Saranac Lake, New York.

[2] This study was supported by research grants E36C12 and E36C13 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, U. S. Public Health Service.

MATERIALS AND METHODS

An effort was made to perform the double-diffusion precipitin technique in accord with the report of Parlett and his co-workers (6); however, information kindly supplied by Dr. Parlett suggested changes from their original procedure. Therefore, it seemed advisable to present a brief description of the double-diffusion technique employed at the Trudeau Laboratory, as it varies slightly from published reports.

Antigens: Culture filtrates of the H37Ra or H37Rv strain of *M. tuberculosis* were used which had been concentrated by pervaporation to 2 per cent of the original volume of Proskauer and Beck fluid synthetic medium. The filtrates were unheated and were sterilized by filtration through sintered glass (ultra-fine porosity), Seitz, or millipore filters, as indicated below. Further comment on the variety of filters employed will be made later in this report. The following lots of antigen were used:

Lot 8-4—H37Rv (eight weeks) sintered glass filtered, dialyzed, and concentrated to 2 per cent of the original volume and preserved with thimerosal (Merthiolate®) at a final concentration of 1:10,000.

Lot 11-3—H37Rv (eight weeks) Seitz filtered. Pervaporation without prior dialysis, as described by Parlett and Youmans (8), resulted in heavy precipitation on concentration, and it was necessary to clarify the filtrate by passage through fine filter paper (Whatman No. 40). The filtrate was then dialyzed and processed in the same manner as Lot 8-4.

Lot 1-6—H37Ra (eight weeks) sintered glass filtered and then dialyzed. Concentrated by pervaporation and finally Seitz filtered and processed in the same manner as Lot 8-4.

Lot 6-17—H37Rv (eight weeks) millipore filtered and then dialyzed. Concentrated by pervaporation, millipore filtered, and stored frozen. Thimerosal to a concentration of 1:10,000 was added before use.

A portion of Lot 8-4 was sent to Dr. Parlett, who found it comparable in activity with the antigens prepared in their laboratory (9). Lots 11-3, 1-6, and 6-17 were subsequently compared at the Trudeau Laboratory with Lot 8-4, using reactive human serum. They were found to be equally sensitive.

Human serum: The specimens of human serum tested in this study were obtained from patients

and employees of two nearby hospitals.[3] With few exceptions, fasting samples were collected. The serum from the clotted blood was collected and stored at −20°C., unless immediately used in one of the serologic tests. No information about the subjects who were bled was furnished to the laboratory until after the serologic tests had been completed. After the diagnoses of the patients were made known, it was found that 12 persons had been bled twice and one person three times. (The data from these subjects are treated separately in table 6 and are not included in table 2, which contains data from persons bled once only.)

Gel precipitin technique: Tubes of soft glass 10 cm. long were prepared, as described by Parlett and co-workers (6), for use in the gel precipitin test. The principal modification of the published technique (6), which was suggested by Dr. Parlett and incorporated in the procedure, was the reduction of the agar reaction arena separating the antigen from the serum to a column 0.5 cm. long. Difco Noble agar was used, and all agar concentrations were prepared in phosphate-buffered saline, pH 7.4, with glycine present in a concentration of 1.0 M. The 1:1 and 2:1 antigen to agar dilutions were prepared with 2 per cent agar, giving respectively 1 per cent and 0.6 per cent final agar concentration in the antigen column. Fifteen or twenty minutes before addition of the serum to be tested, 0.5 cm. of 0.5 per cent agar was layered above the antigen column. The antigen column was 3 cm. in height and the serum column was also 3 cm. These standard measures were adhered to throughout the study in order to facilitate comparison of antigen and antiserum. The tubes were sealed with Parafilm[4] and incubated vertically at 37°C. for a period of twenty-one days. The results of observations were recorded in terms of the distance in millimeters that a precipitin line occurred from the serum-arena interface. The nature of the line was described as diffuse, very fine (VF), or fine (F), and the prefix "D" indicated an intense line. Thus a 2.0 DVF would represent a line at 2 mm. from the serum interface, which was intense and of minimal width. These and other notations helped to form a record of the changes in the individual tubes for future reference. In this report, however, unless otherwise noted, a serum is designated to be positive if a minimum of at least one visible VF or F line had occurred at some time during the twenty-one-day observation period. Only one test at each antigen dilution was performed on the 276 specimens of serum from single bleedings.

Hemagglutination and hemolytic reactions: An aliquot of each serum was prepared for use in the sensitized erythrocyte hemagglutination and hemolytic reactions, which were performed according to a drop modification (10). The antigen employed to sensitize the sheep erythrocytes was a 1:15 dilution of OT (prepared at the Trudeau Laboratory—TOT—and comparable with 4× Lederle OT in serologic activity), or 1:15 dilutions of the concentrated unheated culture filtrates employed in the gel diffusion reactions. Both of the sensitized erythrocyte tests were read following one-half hour of incubation at 37°C., and again after standing overnight at room temperature.

[3] Ray Brook State Tuberculosis Hospital, Ray Brook, New York; and Veterans Administration Hospital, Sunmount, New York.

[4] Parafilm was obtained from the Will Corporation, Rochester, New York.

Results

Gel precipitin and sensitized erythrocyte tests of serum from 276 subjects: The serum specimens tested by the gel precipitin method with each lot of antigen are recorded in table 1. The first 57 specimens received were tested with Lot 8-4, the next 53 with Lot 11-3, et cetera. As stated before, at the time the serum was tested the patient's diagnosis was unknown to the laboratory and, therefore, the distribution of serum tested with each lot was uncontrolled. It may be seen, however, that the cases tested with each antigen were fairly representative of the various diagnoses. Lot 6-17 was prepared when nearly all testing was completed and was employed mainly in comparative tests to be discussed later in this report.

The significant demonstration recorded in table 1 is that each antigen was reactive with both tuberculous and nontuberculous serum. This was not evident in the comparative tests with only a few reactive serum specimens, referred to in the Materials and Methods section. It may be seen that the results summarized in the following tables were not weighted toward a demonstration of specificity or nonspecificity due to marked differences among the antigens employed.

The data obtained when the serum from 276 persons with different diagnoses was examined by both the gel precipitin and erthrocyte sensitization techniques are summarized in table 2.

There was little difference in the number of positive reactions observed in gel at the two different antigen concentrations. However, individual serum varied, so that an occasional specimen was positive at a single antigen dilution.

The number of positive hemagglutination reactions approximated the number of positive gel tests, while fewer hemolytic reactions were noted, although some serum specimens gave positive hemolytic tests only.

The distribution of positive reactions was such that for all cases more serum reacted in both the gel and sensitized erythrocyte tests than in either

TABLE 1

CLASSIFICATION OF CASES AND THE NUMBER OF SERUM SPECIMENS TESTED IN GEL WITH EACH LOT OF ANTIGEN

Diagnosis	Lot 8-4			Lot 11-3			Lot 1-6			Lot 6-17		
	Number of Cases	Positive	Negative	Number of Cases	Positive	Negative	Number of Cases	Positive	Negative	Number of Cases	Positive	Negative
Active tuberculosis												
Far advanced	16	13	3	13	9	4	44	36	8	0	0	0
Moderate	7	3	4	8	3	5	21	7	14	1	0	1
Minimal	2	2	0	3	1	2	5	4	1	0	0	0
Inactive tuberculosis												
Far advanced	6	1	5	0	0	0	8	2	6	0	0	0
Moderate	7	2	5	2	2	0	5	2	3	0	0	0
Minimal	2	0	2	1	0	1	4	1	3	0	0	0
Not rated	0	0	0	0	0	0	4	3	1	0	0	0
Tuberculous activity undetermined	3	1	2	6	1	5	5	1	4	2	2	0
Tuberculosis never proved	4	1	3	2	0	2	0	0	0	0	0	0
Unclassified mycobacterial disease	1	0	1	1	1	0	4	4	0	0	0	0
Pulmonary disease; etiology undetermined	4	2	2	3	0	3	5	2	3	0	0	0
Indefinite diagnosis	2	1	1	2	1	1	2	0	2	1	0	1
Nontuberculous	3	1	2	12	3	9	51	9	42	4	2	2
Total cases	57	27	30	53	21	32	158	71	87	8	4	4

alone. However, this relationship was not true in all diagnostic categories. For example, the serum from 47 of the 73 patients with far advanced active tuberculosis reacted in both tests, while 12 specimens were positive in gel only, and 9 were positive exclusively in the erythrocyte sensitization tests, a total of 68 reactive serum specimens. In contrast, 17 of the 70 nontuberculous patients were serologically positive, but only one had serum that reacted in both the gel and sensitized erythrocyte tests.

Among the nontuberculous subjects were 20 known tuberculin-negative persons, 5 of whom had serum that gave positive gel precipitin reactions, while the serum from 2 was positive in the erythrocyte sensitization tests. Fourteen were negative in both procedures. These data emphasize the nonspecificity of the tests as conducted in this laboratory. The possibility that the positive serologic reactions represent undiagnosed tuberculosis is difficult to support as it credits the tests with greater sensitivity than the negative results with proved cases warrant.

No indication was observed of a greater sensitivity or specificity of the gel precipitin reaction than of the erythrocyte sensitization tests.

Probably the most interesting aspect of the gel precipitin procedure is the resolution of multiple precipitin systems in one test. A number of the gel-positive serum specimens gave more than one definite precipitin line, as may be seen in the tabulation of those specimens in table 3. It is clear that most of these specimens were from patients with far advanced active tuberculosis. Whether or not multiple lines are of particular significance in the present test remains to be established.

Comparative erythrocyte sensitization tests with Old Tuberculin and culture filtrate antigens: In table 1, data were presented indicating comparable gel precipitin reactivity of the first three lots of unheated culture filtrate with serum from subjects in all diagnostic classifications.

At the time when the serum was tested with Trudeau OT-sensitized erythrocytes, as many specimens as the limited supply of antigen would permit were also tested with erythrocytes sensitized with the unheated culture filtrates. One hundred and nine serum specimens were examined, and it may be seen in table 4 that considerable variation in activity of the antigen preparations was demonstrated in the erythro-

cyte sensitization tests. It will be recalled that different methods of filtration were employed in sterilizing the unheated culture filtrates (*see* Materials and Methods). Originally, the intention had been to employ sintered glass filters. However, it soon became apparent that they clogged easily and, therefore, Seitz and eventually millipore filters were used because of the convenient disposable filter pads. One reason for the variation in the erythrocyte sensitization activity of the filtrates is apparently related to the method of filtration, although no influence on the precipitating activity of the filtrates has been noted (11).

Comparative gel precipitin tests with Old Tuberculin and culture filtrate antigens: Old Tuberculin, as routinely prepared, has been reported to be relatively ineffective as an antigen in the gel

TABLE 2

COMPARATIVE REACTIVITY OF 276 SERUM SPECIMENS WHEN TESTED BY THE GEL PRECIPITIN AND ERYTHROCYTE SENSITIZATION TECHNIQUES

Diagnosis	Number of Cases	Positive Serology								All Serology Negative
		Gel*		SR†		Gel Only	SR Only	Gel and SR	Total Positive	
		2:1	1:1	HL	HA					
Active tuberculosis										
Far advanced	73	52	54	39	55	12	9	47	68	5
Moderate	37	9	10	8	12	9	10	4	23	14
Minimal	10	7	7	4	6	1	1	6	8	2
Inactive tuberculosis										
Far advanced	14	3	3	4	5	2	4	2	8	6
Moderate	14	6	5	3	6	1	2	5	8	6
Minimal	7	1	1	3	4	0	4	1	5	2
Not rated	4	3	3	1	2	1	0	2	3	1
Tuberculous activity undetermined	16	4	2	1	3	4	2	1	7	9
Tuberculosis never proved	6	1	1	0	1	1	1	0	2	4
Unclassified mycobacterial disease	6	5	5	2	2	3	1	2	6	0
Pulmonary disease; etiology undetermined	12	4	3	1	2	2	1	2	5	7
Indefinite diagnosis	7	2	1	1	1	2	1	0	3	4
Nontuberculous	70	10	7	0	6	11	5	1	17	53
Total cases	276	107	102	67	105	49	41	73	163	113

* Sera tested in tubes containing 2 parts of antigen to 1 part of agar and 1 part of antigen to 1 part of agar.

† SR—erythrocyte sensitization tests; HL—hemolytic reaction; HA—hemagglutination reaction.

TABLE 3

NUMBER OF SERUM SPECIMENS SHOWING MORE THAN ONE PRECIPITIN LINE IN GEL

Diagnosis	Number of Precipitin Lines					
	2		3		4	
	2:1	1:1	2:1	1:1	2:1	1:1
Far advanced active tuberculosis	16	14	6	3	2	3
Moderately advanced active tuberculosis	4	3	1	1	0	0
Minimal active tuberculosis	0	1	2	0	0	0
Inactive tuberculosis	3	3	2	2	0	0
Tuberculous activity undetermined	1	0	0	0	0	0
Unclassified mycobacterial disease	2	2	0	0	0	0

TABLE 4

OCCURRENCE OF HEMOLYSINS (HL) AND HEMAGGLUTININS (HA) IN 109 CONSECUTIVE SERUM SPECIMENS WHEN TESTED WITH FOUR ANTIGENS

Diagnosis	Total Number of Cases	Positive Reactions—Sheep Erythrocytes Sensitized with:								Negative in All Tests
		TOT* 1:15		8-4† 1:15		11-3† 1:15		1-6† 1:15		
		HL	HA	HL	HA	HL	HA	HL	HA	
Active tuberculosis										
Far advanced	29	12	21	14	12	1	0	5	10	7
Moderate	15	2	3	1	1	0	0	0	2	11
Minimal	5	1	2	0	1	1	1	0	1	3
Inactive tuberculosis										
Far advanced	6	1	0	2	1	0	0	0	0	3
Moderate	9	1	4	2	3	0	0	0	0	4
Minimal	3	2	1	2	1	0	0	1	0	1
Not rated	0	0	0	0	0	0	0	0	0	0
Tuberculous activity undetermined	9	0	2	1	1	0	0	0	1	6
Tuberculosis never proved	6	0	1	1	1	0	0	0	0	5
Unclassified mycobacterial undisease	2	0	1	0	0	0	0	0	0	1
Pulmonary disease; etiology undetermined	7	1	2	2	1	0	0	0	0	3
Indefinite diagnosis	3	0	0	1	0	0	0	0	0	2
Nontuberculous	15	0	2	0	1	0	0	0	1	13
Total cases	109	20	39	26	23	2	1	6	15	59

* TOT—Trudeau Old Tuberculin.
† Lot of antigen used and dilution.

precipitin test with human serum. Burrell and his co-workers (7) tested forty serum specimens from tuberculous patients by the gel precipitin method, using Lederle OT as the antigen, and observed only five positive reactions, although thirty-two reacted in the erythrocyte sensitization tests. Parlett and Youmans (12) reported that OT was poorly reactive in the gel precipitin test, and hypothesized that the gel diffusion antigens were probably denatured in the process of tuberculin preparation. In neither of these studies was the OT used at the same concentration as the unheated filtrates used by Parlett and his associates (6) or in the present investigation. Therefore, an aliquot of Trudeau OT was concentrated to the same fraction (2 per cent) of its original culture volume as the unheated filtrates previously described. This was then used as the antigen in gel precipitin tests of 20 serum specimens selected on the basis of their reactivity with unheated culture filtrate antigens and before the patient's diagnosis was known.

The results of this comparative test are shown in table 5, in which it may be seen that the concentrated Trudeau OT gave positive reactions with 14 of the 20 specimens tested. Of the 6 specimens with which Trudeau OT did not react, 4 were found to be from the nontuberculous patients or those with inactive disease. The other 2, specimens 91 and 161, were from patients with active disease. These specimens did not give particularly striking or early reactions with the unheated antigens. The data suggest that, with some serum specimens, concentrated Trudeau OT may be as reactive as unheated culture filtrates, and are in disagreement with the contention that OT is poorly reactive in gel precipitin tests (12).

Comparative gel precipitin reactions with unheated culture filtrate antigens: After the identity of the patients was made known, it was decided to retest the paired serum specimens (referred to in Materials and Methods) with Lot 6-17 antigen in order to provide a set of data on the reproducibility of results with serum from consecutive bleedings when tested with unheated culture filtrate antigens. All of the serum had been frozen,

some for several months, and only 2 specimens had been tested originally with Lot 6-17.

Serum from 7 of the 12 subjects who were bled serially did not react in the gel precipitin test with any antigen in either bleeding. Four of the nonreactive subjects were diagnosed as nontuberculous, one was a minimal inactive case, one a minimal active case of tuberculosis, and the remaining person had extrapulmonary tuberculosis. These cases have been omitted from table 6 in order to save space. Seven specimens from patients with far advanced active disease were retested and included in table 6 for further illustration of the comparative activity of different lots of antigen.

The variations observed when the serum was tested are obvious. The interesting aspect of the data is the similarity displayed in many instances. For example, serum obtained on February 11, 1959, from *Case No. 149* showed a delayed response compared with the serum from the other two bleedings when tested with Lot 1-6 antigen. The same pattern is evident when the serum was tested with Lot 6-17 antigen. An absence or delay in response in the original test was generally reproduced, as may be seen when serum from *Cases 129*, *108*, and *204* was retested.

The discrepancies between the number of lines demonstrated with the different antigens at the two dilutions employed are overemphasized to some degree. The number of lines recorded are the number that could be differentiated clearly. In many instances, two or more lines were probable, but both did not become well defined and, therefore, are not noted in the summary data.

The position of the precipitin bands in the majority of reactions recorded in this report was about 2 mm. from the serum interface. In tests

TABLE 5

THE COMPARATIVE REACTIVITY OF CONCENTRATED OLD TUBERCULIN AND UNHEATED CONCENTRATED CULTURE FILTRATES IN GEL PRECIPITIN REACTIONS

Case Number	Diagnosis	Antigen Lot	Unheated Filtrates				Old Tuberculin			
			Day Rated Positive at Antigen Dilution		Maximal Number of Lines at Antigen Dilution		Day Rated Positive at Antigen Dilution		Maximal Number of Lines at Antigen Dilution	
			2:1	1:1	2:1	1:1	2:1	1:1	2:1	1:1
	Active tuberculosis									
27	Far advanced	8-4	2	—	2	—	6	2	1	1
86		11-3	2	2	2	1	1	1	2	2
91		11-3	10	8	1	1	—	—	—	—
95		11-3	6	6	1	1	5	5	1	1
99		11-3	2	2	2	1	1	2	2	2
101		11-3	2	2	1	1	2	2	1	1
136		1-6	3	3	2	2	5	13	1	1
164		1-6	7	4	2	1	2	3	2	1
161	Moderate	1-6	—	4	—	1	—	—	—	—
180		1-6	7	3	1	1	3	3	1	1
	Inactive tuberculosis									
44	Far advanced	8-4	17	—	1	—	—	—	—	—
46	Moderate	8-4	8	8	2	2	6	2	1	1
108	Minimal	11-3	—	8	—	1	—	—	—	—
154		1-6	3	4	1	1	4	1	1	1
92	Unclassified mycobacterial disease	11-3	1	1	2	2	1	2	1	1
170		1-6	3	—	1	—	2	2	1	1
68	Nontuberculous	11-3	7	—	2	—	6	3	1	1
106		11-3	8	12	1	2	—	—	—	—
166		1-6	5	—	1	—	—	—	—	—
	Pooled sera	8-4	6	3	1	1	—	5	—	1

TABLE 6

THE COMPARATIVE REACTIVITY OF UNHEATED CULTURE FILTRATES WITH SELECTED HUMAN SERUM

Case Number	Diagnosis	Date Bled	Antigen Lot	Day Rated Positive at Antigen Dilution		Maximal Number of Lines at Antigen Dilution		Retest Antigen Lot	Day Rated Positive at Antigen Dilution		Maximal Number of Lines at Antigen Dilution	
				2:1	1:1	2:1	1:1		2:1	1:1	2:1	1:1
	Active tuberculosis											
149	Moderate	1-28	1-6	4	—	1	—	6-17	2	4	1	1
		2-11	1-6	7	7	1	1	6-17	4	4	1	2
		3-11	1-6	3	3	2	1	6-17	2	Not done	2	Not done
	Inactive tuberculosis											
204	Moderate	2-18	1-6	—	—	—	—	6-17	—	—	—	—
		3-11	1-6	14	—	1	—	Not done				
258	Minimal	3-11	1-6	1	2	2	1	6-17	1	1	2	2
		3-18	1-6	2	1	1	2	6-17	4	1	1	2
108		1-14	11-3	—	8	—	1	6-17	—	—	—	—
		1-22	1-6	—	—	—	—	6-17	—	—	—	—
129	Nontuberculous	1-22	1-6	—	—	—	—	6-17	—	—	—	—
		2-11	1-6	—	—	—	—	6-17	6	7	1	1
	Active tuberculosis											
48	Far advanced		8-4	1	1	1	1	6-17	1	1	3	2
86			11-3	2	2	2	1	6-17	1	1	2	2
88			11-3	2	2	1	1	6-17	2	1	2	2
212			1-6	2	1	4	4	6-17	1	1	5	2
214			1-6	1	1	2	2	6-17	1	1	1	2
235			1-6	4	5	1	2	6-17	4	4	1	1
238			1-6	1	1	2	2	6-17	2	2	2	3

in which multiple lines occurred, as with *Case No. 212* (table 6), one or two lines appeared at the serum interface. The remaining lines were generally grouped in the column 2 or 3 mm. from the serum interface.

DISCUSSION

The data indicate that, in its present form, the double-diffusion gel precipitin method is no more specific in the detection of antibody in the serum of tuberculous patients than is the hemagglutination reaction. Combining the results of the two methods raises the number of both positive and false-positive reactions detected, since the methods lack specificity while apparently measuring different antibody responses. The latter observation has been noted with rabbit and human serum by Rheins and his associates (7, 13). In their report, the reactivity of the human serum from tuberculous patients was much lower than found in the present study. They employed diluted Lederle OT as antigen and may, therefore, have had an inadequate antigen concentration.

The results of both the erythrocyte sensitization tests and the gel precipitin reactions are consistent with the demonstration of cross-reactive components in culture filtrates of the mycobacteria. Lagercrantz (14) has found that "virulent and avirulent mycobacteria, various strains of staphylococci and some strains of fungi evoked significant antibody response against erythrocyte-adsorbable antigen from the tubercle bacillus." Adsorption and inhibition reactions were then employed to show that the response was not entirely nonspecific. It is not surprising, in view of this, that serum from nontuberculous subjects often reacts with OT-sensitized erythrocytes.

The experimental demonstration of cross reactions in gel precipitin tests between mycobacterial culture filtrates and antiserum to antigens from nonmycobacterial sources remains to be shown. However, considering the results obtained

with human serum, there is no reason to doubt that this will be done.

Summary

Serum from 276 tuberculous and nontuberculous subjects was tested for antibody by the double-diffusion gel precipitin technique. Unheated, concentrated culture filtrates of the H37Ra or H37Rv strains of tubercle bacilli were employed as antigens. Fifty-nine of 73 specimens of serum (81 per cent) from cases of active far advanced tuberculosis and 79 of 120 specimens (66 per cent) from all cases of active tuberculosis tested gave positive gel precipitin reactions. Seventeen per cent of 70 specimens of serum from nontuberculous subjects gave positive reactions.

Approximately the same number of positive reactions occurred when the serum was tested with sheep erythrocytes sensitized with Trudeau OT diluted 1:15, although the same serum specimens did not necessarily react in both tests.

Comparative tests demonstrated that the different antigen preparations were comparable in gel precipitin reactivity, but varied in their erythrocyte-sensitizing activity.

The results are considered to be consistent with the known presence of cross-reactive antigens in mycobacterial culture filtrates.

Sumario

Comparación de las Técnicas del Gel de Precipitina y de los Eritrocitos Sensibilizados para el Descubrimiento de Anticuerpo en el Suero de los Tuberculosos y los No Tuberculosos

Los sueros de 276 sujetos tuberculosos y no tuberculosos fueron comprobados en cuanto a anticuerpos con la técnica de doble difusión del gel de precipitina. Como antígenos, se emplearon filtrados culturales concentrados de las cepas H37Ra o H37Rv de bacilos tuberculosos. Acusaron reacciones positivas al gel de precipitina 59 de 73 (81 por ciento) ejemplares de suero procedente de casos de tuberculosis muy avanzada activa y 79 de 120 ejemplares (66 por ciento) procedentes de todos los casos de tuberculosis activa comprobados. También acusaron reacciones positivas 17 por ciento de 70 ejemplares de suero procedente de sujetos no tuberculosos.

Hubo aproximadamente el mismo número de reacciones positivas al comprobar el suero con eritrocitos ovinos sensibilizados con la TA de Trudeau diluída al 1:15, aunque no fueron forzosamente los mismos ejemplares los que reaccionaron en ambas pruebas.

Pruebas comparativas demostraron que las diversas preparaciones de antígeno eran comparables en su reactividad al gel de precipitina, pero variaban en su actividad para los eritrocitos sensibilizados.

Se considera que los resultados son compatibles con la presencia conocida de antígenos de reactividad cruzada en los filtrados de cultivos micobacterianos.

Resume

Comparaison des méthodes utilisant des gel-précipitines et celles utilisant des érythrocytes sensibilisés, dans la recherche d'anticorps dans le sérum de malades tuberculeux et non tuberculeux

Les auteurs ont étudié le sérum de 276 sujets tuberculeux et non tuberculeux à la recherche d'anticorps, par la méthode utilisant la double diffusion de gel-précipitines. Ils employèrent comme antigènes des filtrats de cultures de souches H37Ra ou H37Rv de bacilles tuberculeux, non chauffés et concentrés. Cinquante neuf des 73 spécimens de sérum (81%) des cas de tuberculose trés avançée et 79 de 120 spécimens (66%) de tous les cas de tuberculose active donnèrent une réponse positive avec les gel-précipitines. Dix-sept pour cent des 70 spécimens de sérum de sujets non tuberculeux donnèrent des réactions positives.

Il y eut à peu prés le même nombre de réactions positives quand le sérum fut étudié avec des érythrocytes de mouton, sensibilisés avec Trudeau OT à la dilution de 1:15, bien que les mêmes spécimens de sérum n'aient pas réagi nécessairement dans les deux examens.

Dans les examens de comparaison ils démontrèrent que les différentes préparations d'antigènes furent comparables dans leur réactions aux gel-précipitines mais furent différentes dans la sensibilisation des érythrocytes.

Les auteurs pensent que les résultats dépendent de la présence reconnue d'antigènes donnant des contre-réactions dans le filtrat des cultures de mycobactéries.

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A STUDY OF THE DOUBLE-DIFFUSION GEL PRECIPITATION TEST IN TUBERCULOUS PATIENTS[1]

With Special Reference to Technical Problems

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INTRODUCTION

The precipitin test carried out in a gel as originally devised by Oudin (1) has been the subject of a number of modifications and applications. Parlett's double-diffusion gel test has given interesting results in the study of the antigens and antibodies related to mycobacteria. The use of this test was initiated at Herman Kiefer Hospital in 1958, proceeding from the knowledge that existed at the time of the report of Parlett, Youmans, Rehr, and Lester (2).

MATERIALS AND METHODS

Antigens: Each batch of antigen used in this study was prepared in accordance with details outlined by Parlett and Youmans (3). Proskauer and Beck synthetic medium was used to propagate an H37Ra strain of *M. tuberculosis*. The cultures were allowed to grow in pellicle form for three months at 37.5°C. At the end of the growth period, cultures were first passed through a Seitz filter sheet of K-5 porosity to remove the cellular debris and then through a sheet of S-1 porosity to yield sterile culture filtrates.

These filtrates were then placed in sterile Visking cellulose tubing in front of an electric fan and allowed to "pervaporate" down to 15 per cent of the original volume. The concentrated filtrates were again passed through a Seitz filter sheet of S-1 porosity and stored at refrigerator temperature.

Serum: All of the serum tested for precipitating antibody against the H37Ra strain of *M. tuberculosis* was obtained from employees and patients of the Herman Kiefer Hospital. The blood was drawn under aseptic conditions while the subject was in the fasting state, and the serum was removed promptly to avoid hemolysis. The serum was not inactivated in this study.

Control groups: One group of serum specimens was obtained from 26 employees of the Herman Kiefer Hospital, a second group from women visiting the obstetrical clinic of the hospital, and a third from the Herman Kiefer Hospital blood bank. All of the serum was drawn by laboratory personnel from a section of the laboratory other than the one in which the gel-diffusion tests were performed.

The tube agar double-diffusion test: The test was performed essentially as recommended by Dr. Parlett, except that six instead of two dilutions of antigen were used. Difco purified agar was found to give uniformly clear solutions and gels when prepared with 1 M. aminoacetic acid in a phosphate buffer at pH 7.2 (4).

The 2 per cent and 1 per cent agar reagent solutions were prepared in 1,000-ml. volumes and were of the following composition.

The 2 per cent buffered agar solution employed to make the various dilutions of antigen was prepared as follows:

Aminoacetic acid	75 gm.
Difco purified agar	20 gm.
0.1 N. NaOH	350 ml.
0.1 M. KH_2PO_4	500 ml.
Distilled water	150 ml.

Solution of the ingredients was effected by heating in a boiling water bath and was maintained in the fluid state by means of a 48°C. water bath. Buffered agar solution used in the neutral agar reaction zone was prepared in the same manner but as a 1 per cent agar solution. Screw-cap tubes 20 mm. × 125 mm. were used for storage of the 2 per cent agar, and later served to prepare the antigen-agar dilutions.

Appropriate amounts of the fluid 2 per cent agar were distributed to the screw-cap tubes in the following manner:

Ratio of Antigen/Agar	Agar 2 Per Cent
2:1	1.0 ml.
1:1	1.5 ml.
1:3	2.0 ml.
1:4	2.25 ml.
1:6	2.5 ml.
1:8	2.63 ml.

The 1 per cent buffered agar solution was likewise distributed in 10-ml. amounts in screw-cap tubes. The tubes now containing the appropriate amounts of agar solutions were sterilized in the autoclave at 8 pounds per square inch for ten minutes. Extreme caution must be exercised in sterilizing the agar solutions because the aminoacetic acid

[1] From the Tuberculosis Division, Herman Kiefer Hospital; and the Bureau of Laboratories, Detroit Health Department, Detroit, Michigan.

breaks down at high temperatures and results in brown solutions which are unsatisfactory.

Preparation of capillary tubes: Internal coating of the 10 cm. long × 3 mm. internal diameter tubes[2] was accomplished as follows: Clean, new tubes as received were inverted in a beaker containing 1 per cent melted agar prepared with distilled water. The beaker was covered and autoclaved at 15 pounds per square inch for fifteen minutes. After the beaker of agar and tubes had cooled sufficiently, but while the agar was still fluid, the tubes were emptied by shaking out the liquid. The tubes were then dried in a 56°C. air oven overnight and stored in a covered container in a 37°C. bacteriologic incubator.

The preparation of antigen/agar mixtures: The antigen was used as taken from the bottle and warmed in the water bath 45–48°C. The sterile 2 per cent buffered agar which had previously been prepared and distributed in variable amounts was likewise melted and maintained in the water bath. The antigen/agar mixtures were prepared as follows:

Antigen/Agar	Antigen	Agar 2
		Per Cent
2:1	2.0 ml.	1.0 ml.
1:1	1.5 ml.	1.5 ml.
1:3	1.0 ml.	2.0 ml.
1:4	0.75 ml.	2.25 ml.
1:6	0.5 ml.	2.5 ml.
1:8	0.37 ml.	2.63 ml.

From each antigen/agar mixture tube, 10 gel-diffusion capillary tubes were prepared, the antigen/agar mixture being introduced into the bottom of the capillary tubes to a depth of 2.5 cm. by means of a sterile opsonic pipette with a 10-cm. long delivery tip.

To perform the test: A second layer of fluid 1 per cent buffered agar was placed 0.5 cm. deep over the antigen layer, and the tubes were placed in the refrigerator to gel. The tubes were now ready to receive the sterile undiluted serum which was introduced into the gel-diffusion tubes by means of another sterile opsonic pipette.

The filled reaction tubes were capped by rubber bulbs (supplied with smallpox-vaccinating outfits) to prevent evaporation of the serum during incubation. Blocks of wood 7- × 3- × 1-inch with holes to support the tubes in a vertical position were a convenience in handling and reading. The tubes were incubated at 37°C. and examined at twenty-four-hour intervals for a period of five days. As soon as a band or bands of precipitate were observed in any one of the tubes, the serum was recorded as positive for tuberculous antibody. No attempt was made at quantitation in this study, the tests being read as positive or negative.

The precipitate is most easily seen in subdued room light with the tubes transilluminated by a concentrated light source.[3] Viewed from the proper angle, the bands appear quite dense and of appreciable width. Extremely fine lines that occasionally appear should be distinguished from the true bands of precipitation. These do not migrate, do not increase in density with prolonged incubation, and are thought to be nonspecific; hence, are to be disregarded. The preliminary internal coating of the tubes with agar was thought to reduce the frequency of their appearance.

[2] Aloe Scientific Co.

[3] The model 369 American Optical Co. microscope lamp works well.

Results

After preliminary work on the production of antigen which was standardized against rabbit serum and against serum from cases of known active tuberculosis in parallel with antigen furnished by Dr. Parlett, a product was obtained which seemed to be satisfactory and was available in sufficient quantity for large-scale testing of hospital patients. The tests were carried out blindly as far as the laboratory was concerned, using the original technique outlined by Parlett with an antigen designated as No. 3.

Of 885 patients, only 17 per cent with pulmonary tuberculosis and 25 per cent with extrapulmonary tuberculosis had serum that gave positive reactions. This poor result turned attention to the details of technique, and it was found that all batches of antigen did not behave in the same manner. It also became apparent that the zone phenomenon was involved in the production of false negative tests. Consequently, selected previously negative serum (preserved by freezing) was retested with different batches of antigen and the dilutions of antigen were gradually increased until the technique outlined above under "Material and Methods" was settled upon as

TABLE 1

Group I: 40 Patients Tested with H37Ra Antigen, Lot No. 5

	Positive	Negative
Far advanced tuberculosis	21	1*
Moderately advanced tuberculosis	12	0
Minimal tuberculosis	3	0
Pleural effusions	1	1†
Cervical adenitis	1	0

* A 22-year-old white male treated for two years; sputum became noninfectious. This was probably an arrested case.

† A 31-year-old Negro male, tuberculin positive; kept under observation and treatment; nine months later tuberculosis was not proved. Completely cleared under antituberculous therapy.

adequate to reveal reactivity in a wide range of titer. The effectiveness of the test then assumed an entirely different aspect. The results in 136 cases appear in tables 1, 2, 3, and 4, which were prepared retrospectively after trial and error testing but without elimination of any of the serum submitted.

There were four negative tests. One, on the serum of a patient who was probably nontuberculous from the beginning, is listed in table 1. In table 2 a similar case is listed as well as that of a patient who had had tuberculosis previously but had returned to the hospital under an alias and had been studied as a new case. Instances of patients who had responded well to treatment are illustrated in tables 1 and 4. The other tests were all positive and occurred in patients whose original diagnoses of active disease needed no revision. The positivity rate, regardless of the classification of disease on the basis of extent and location, thus approached 98 per cent.

Control groups were employed chiefly as a check on the consistency of the technique. The number is too small for any other purpose. However, one special group furnished surprising and highly significant findings.

Group I, tested with antigen No. 6: Twenty-six hospital employees were tested incidental to their periodic physical examination. All were known to have positive Mantoux reactions, but chest films negative for tuberculosis. All were in good health and were working. Serum from exactly half of the group gave positive reactions to the gel diffusion test; most of the reactions were strong, with four bands of precipitation. Of the 13 reactors, 12 worked with tuberculous patients, and one worked in the obstetrical department. Among the 13 nonreactors, 6 were employed in tuberculosis areas and 7 in nontuberculous disease areas of the hospital.

Group II, tested with antigens Nos. 6 and 8: Thirty obstetrical patients were selected at random in the clinic. The serum from all was negative for antibody. These patients came from an area of the city where the incidence of tuberculosis is highest.

Group III, tested with antigen No. 6: The 17 subjects were all blood donors and the serum of all was negative for antibody. Most of these were relatives or friends of patients with tuberculosis.

Biologic false positives: Among 17 apparently nontuberculous patients, who for a variety of reasons were admitted to the tuberculosis wards, 2 had serum that gave positive reactions. This result was obtained early in the work with antigen No. 3, and probably the number should have been greater. However, in a tuberculosis environment, clear proof of a biologic false positive is difficult to establish. Experience thus far is in accord with Dr. Parlett's study which indicates that biologic false positives are rare (5).

TABLE 2

GROUP II: 62 PATIENTS WITH H37Ra ANTIGEN, LOT NO. 6

	Positive	Negative
Far advanced tuberculosis	23	0
Moderately advanced tuberculosis	29	1*
Minimal tuberculosis	1	0
Pleural effusion	8	1†

* After 7 months of observation and treatment, tuberculosis not proved; no roentgenographic change in lesion.

† Found to be a former patient; unchanged chest films since 1929; inactive case.

TABLE 3

GROUP III: 26 PATIENTS WITH H37Ra ANTIGEN, LOT NO. 8

	Positive	Negative
Far advanced tuberculosis	15	0
Moderately advanced tuberculosis	8	0
Minimal tuberculosis	3	0

TABLE 4

GROUP IV: 6 PATIENTS WITH H37Ra ANTIGENS, NOS. 5, 6, 7

Results with all lots the same

	Positive	Negative
Far advanced tuberculosis	4	1*
Pleural effusion	1	0

* 38-year-old-Negro male, treated for 5 months; sputum noninfectious at time of test.

SUMMARY

The double-diffusion gel precipitin test for the antibodies that are a response to *Mycobacterium tuberculosis* possesses a high degree of sensitivity provided the full effectiveness of the test is ex-

ploited by the use of multiple antigens in serial dilution.

A satisfactory method of standardizing antigen has not been found. Consequently, to avoid false negative reactions, it seems necessary to check these against more than one batch of antigen and at the same time employ the antigen in serial dilution. (More simple solutions readily come to mind but it seemed undesirable to alter the basic technique in the course of an initial study.)

In its present state of development, the test can hardly be considered a quantitative one and the sensitivity is such that it furnishes little information about the extent or activity of the disease. Moreover, the immune response that results from continuous exposure to tuberculosis can be of the same degree as that which results from active disease. This study provides no precise information regarding the disappearance of antibody after arrest or complete healing beyond the fact that negative reactions do occur in former tuberculous patients with noninfectious sputum.

The healthy tuberculin-positive person can be expected to react negatively to the gel diffusion test. It follows that a positive test means active disease except in the special situation already noted, namely, long continuous exposure to tuberculosis.

Experience up to the present time—and this is only preliminary—indicates that when interpreted with circumspection the gel diffusion test can be of critical value in distinguishing tuberculosis from nontuberculous disease.

Sumario

Estudio de la Prueba de Precipitación del Gel de Doble Difusión en los Tuberculosos con Referencia Particular a los Problemas Técnicos

La prueba de la doble difusión de las precipitinas del gel para los anticuerpos que constituyen una respuesta al *Mycobacterium tuberculosis* posee una sensibilidad de alto grado con tal que se aproveche la plena eficacia de la prueba con el uso de antígenos múltiples en diluciones seriadas.

No se ha encontrado todavía un método satisfactorio para normalizar el antígeno. Por consiguiente, para evitar seudonegativas, parece necesario comprobar éstas con más de un lote de antígeno, empleando al mismo tiempo el antígeno en dilución seriada. (Acuden fácilmente a la mente soluciones más sencillas, pero pareció contraproducente alterar la técnica fundamental en el transcurso de un estudio inicial.)

En su actual etapa de desenvolvimiento, apenas cabe considerar a la prueba como cuantitativa, y es tal su sensibilidad que aporta poca información acerca de la extensión o actividad de la dolencia. Además, la inmunirreacción proveniente de la continua exposición a la tuberculosis puede alcanzar la misma intensidad que la proveniente de la enfermedad activa. Este estudio no suministra información precisa con respecto a la desaparición de los anticuerpos a continuación del estacionamiento o cicatrización total aparte del hecho de que sobrevienen reacciones negativas en los casos antiguos cuando el esputo es negativo para bacilos tuberculosos.

Cabe esperar que la persona tuberculino-positiva sana reaccione negativamente a la prueba de difusión del gel. De esto se desprende que una prueba positiva denota enfermedad activa excepto en la situación especial ya señalada, a saber, prolongada y continua exposición a la tuberculosis.

Lo observado hasta ahora, y esto es puramente preliminar, indica que, interpretada con circunspección, la prueba de difusión del gel puede ser de valor decisivo para distinguir la enfermedad que es tuberculosa de la que no lo es.

Resume

Etude de la réaction de diffusion double des "gelprécipitines" chez des malades tuberculeux, portant spécialement sur les problèmes techniques

La réaction de diffusion double des "gel-précipitines" mettant en jeu les anticorps qui répondent au *Mycobacterium tuberculosis* possède une sensibilité fidèle pourvu que le plein effet de la réaction soit exploité par l'emploi d'antigènes multiples dans des séries de dilutions.

On n'a pas encore trouvé une méthode satisfaisante pour standardiser les antigènes. Par conséquent pour éviter des réactions faussement négatives, il semble nécessaire de vérifier celles-ci dans plus d'un lot d'antigènes et d'employer en même temps les antigènes dans des séries de dilutions. (Des solutions plus faciles viennent aisément à l'esprit mais il semble indésirable de changer la technique de base au cours d'une première étude.)

Dans ses conditions actuelles de développement, cette réaction peut être difficilement considérée comme quantitative et sa sensibilité est telle qu'elle fournit peu d' information sur l'activité ou l'extension de la maladie. En plus, la résponse d'immunité qui résulte d'une exposition continue à la tuberculose peut être du même degré que celle qui résulte d'une maladie active. Cette étude

ne donne pas d'information précise en ce qui concerne la disparition des anticorps après l'arrêt d'extension de la maladie ou après une complète cicatrisation, sans compter le fait que des réactions négatives se produisent chez les cas anciens, quand l'expectoration ne contient pas de bacilles tuberculeux.

Le sujet sain avec une réaction positive à la tuberculine doit réagir d'une façon négative à la réaction de diffusion. Il s'en suit qu'une réaction positive signifie forme active excepté dans la situation spéciale déjà mentionnée, c'est à dire dans le cas d'une exposition longue et continue à la tuberculose.

Jusqu'à présent, l'expérience—qui en est seulement au stade préliminaire—indique que, lorsque la réaction de diffusion des gel-précipitines est interprêtée avec circonspection, elle peut avoir une réelle valeur pour distinguer une affection tuberculeuse d'une affection non tuberculeuse.

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A STUDY OF PULMONARY HISTOPLASMOSIS WITH PARTICULAR REFERENCE TO PNEUMOPERITONEUM THERAPY[1]

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Introduction

The importance of a bacteriologic diagnosis in pulmonary disease cannot be overstressed. The mycoses are playing an increasingly important role and histoplasmosis deserves a special note because the symptoms, signs, and roentgenographic findings may be very similar to those found in pulmonary tuberculosis. The writers have seen several patients with histoplasmosis in whom a pathologic diagnosis of tuberculosis was made in the absence of sputum findings but, on further examination, the tissue changes were shown to have been histoplasmosis (1). Much emphasis has been placed on the fact that many cases of fungous disease are being erroneously treated as "tuberculosis" in the absence of bacteriologic proof. It has been estimated (2, 3) that tuberculosis hospitals throughout the country may contain as many as three thousand cases of histoplasmosis at the present time.

The present report deals with 7 patients with pulmonary histoplasmosis whose mycotic cavities failed to respond to pneumoperitoneum. The case of one of the patients who had been previously treated for pulmonary tuberculosis is presented in considerable detail. Four of the patients were treated as tuberculous with drugs and pneumoperitoneum before the diagnosis of histoplasmosis was established, and the remainder with pneumoperitoneum as the primary treatment of histoplasmosis. From this small series the impression has been gained that tuberculous cavities respond better to collapse measures than the cavities produced by Histoplasma.

Observations

In table 1 the data on the 7 patients treated with pneumoperitoneum since 1954 may be seen. The diagnosis in 3 patients was proved by identification of the Histoplasma, and all had positive complement-fixation reactions. In most instances, positive skin reactions were also present. In the remaining 4 patients, the sputum examinations were negative for fungi. One patient (*Case No. 6*) was proved at this hospital to have both active pulmonary tuberculosis and active histoplasmosis. The sputum from this patient is now negative for tubercle bacilli, and more recent specimens were also negative for Histoplasma. He was treated with extensive antituberculous therapy, as well as a four-month course of amphotericin B by the intravenous route. A cavity in the left apex persists, although the lesion is greatly improved as compared with its appearance on the admission chest film. Resection was advised, but the patient declined to accept surgery at this time.

Another case has been excluded because the diagnosis of aspergillosis was established postoperatively. In this patient, a cavity had failed to respond to pneumoperitoneum and pneumothorax therapy and subsequently appeared to become inspissated. The surgical specimen showed the cavity to contain an abundant growth of the fungus.

Unilateral or bilateral cavitation was present in each case and persisted in spite of pneumoperitoneum and other therapy. Collapse therapy was given for periods ranging from four to thirty-three months. As in each case the cavity or cavities persisted either unchanged or slightly smaller, it would seem that collapse measures such as pneumoperitoneum do not have a favorable effect on mycotic cavities. This may be of importance because of the planning of therapy and because surgery may have to be anticipated.

C. B., a 37-year-old white male, was first admitted to this institution in July, 1954. His occupation was that of a farm laborer. His symptoms dated back to January of the same year, namely, loss of weight, malaise, and hemoptysis. A roentgenogram of the chest showed a cavity in the left upper lobe, and one sputum examination prior to hospital admission was reported as having been positive for acid-fast bacilli on microscopy. This was not confirmed by culture.

The patient was hospitalized for 323 days and

[1] From District Two State Tuberculosis Hospital, Louisville, Kentucky.

TABLE 1

RESPONSE OF HISTOPLASMAL CAVITIES TO TEMPORARY COLLAPSE PROCEDURES

Case Number	Duration of Collapse Therapy	*H. Capsulatum* Present	Skin Sensitivity		Complement Fixation Reaction	Cavitation	
			Tuberculin	Histoplasmin		Maximal Diameter	Variations with Therapy
	months					*cm.*	
1	8+	−	+*	+	1/16	6	Smaller
2	17	+	+	+	1/64	5	Smaller
3	10+	−	†	+	1/32–1/64	3	Unchanged
4	4+	−	+	+	1/8–1/64	4	Unchanged
5	28	−	−‡	+	1/32–1/64	2	Unchanged
6	15+	+	+	+	1/128–1/32	7	Smaller
7	33	+	−	+	1/32–1/16	4	Apparently closed

* + = positive.
† not recorded.
‡ − = negative.

treated as "tuberculous," that is, by bed rest, pneumoperitoneum, and chemotherapy consisting of daily isoniazid and intermittent streptomycin. Throughout his stay in the hospital, sputum examinations were negative for tubercle bacilli by microscopy and culture. Subsequent follow-up roentgenograms showed remarkable improvement and the cavity appeared to have closed. He was discharged to continue treatment on an outpatient basis. Chemotherapy and pneumoperitoneum were continued until July, 1957. Both treatments were then abandoned as the patient's condition had remained quite satisfactory throughout regular outpatient check-ups. However, within some months there was recurrence of cavitation in the left upper lobe with bronchogenic spread of the disease to both lower lobes. Tomography showed the cavity quite clearly, and the patient was rehospitalized in November, 1958. He had been working very hard but had complained of no symptoms.

The previous apparent closure of the cavity was doubted in view of such a rapid relapse and the fact that no tomograms had been obtained at the time of his discharge. The original diagnosis of active pulmonary tuberculosis was also questioned at this time because of the sequence of events and because sputum remained negative for tubercle bacilli on repeated microscopic and cultural examinations. During this admission the skin sensitivity reaction was found negative for tuberculin, coccidioidin, and blastomycin, but positive for histoplasmin. The complement-fixation reaction was reported positive for Histoplasma (1:32 mycelial phase, 1:16 yeast phase antigen). Culture reports for fungi were returned eventually, and at least six cultures have been reported positive for *Histoplasma capsulatum*. The relevant roentgenograms from the time of diagnosis in 1954 to the present stage following surgery may be seen in figures 1*A–F*.

Treatment with amphotericin B was commenced prior to surgery; however, this made no impression on the cavitary disease. After three weeks of therapy, a resection of the apical posterior segment of the left upper lobe was carried out. At surgery numerous pleural adhesions were found. The resected specimen contained a cavity about 3 cm. in diameter, the wall being about 3 mm. thick and rather hard in places. Material taken from the surgical specimen was negative for Histoplasma by microscopy and culture. Material was also injected into mice and failed to produce disease. Sections revealed granulation tissue, fibrosis, and inflammatory reaction, but no *H. capsulatum* could be demonstrated. It is presumed that the preoperative treatment had eliminated all of the organisms. Further postoperative treatment with amphotericin B was given as a precautionary measure. The course was uneventful and the patient has been discharged. At his last outpatient check-up in November, 1959, the patient reported that he was feeling well and was symptom-free. His chest roentgenogram is essentially normal apart from evidence of surgery on the left side.

DISCUSSION

The attention of physicians in general is increasingly focused on the pulmonary mycoses, and particularly on histoplasmosis because of its prevalence in the United States, although cases have been reported from other countries (4–6). Histoplasmosis has been reported in about twenty countries throughout the world. In this country the frequency of infection varies from state to state, and recent studies have shown that Kentucky has a histoplasmin sensitivity rate of more than 80 per cent. Treatment with amphotericin B and other drugs is at present being

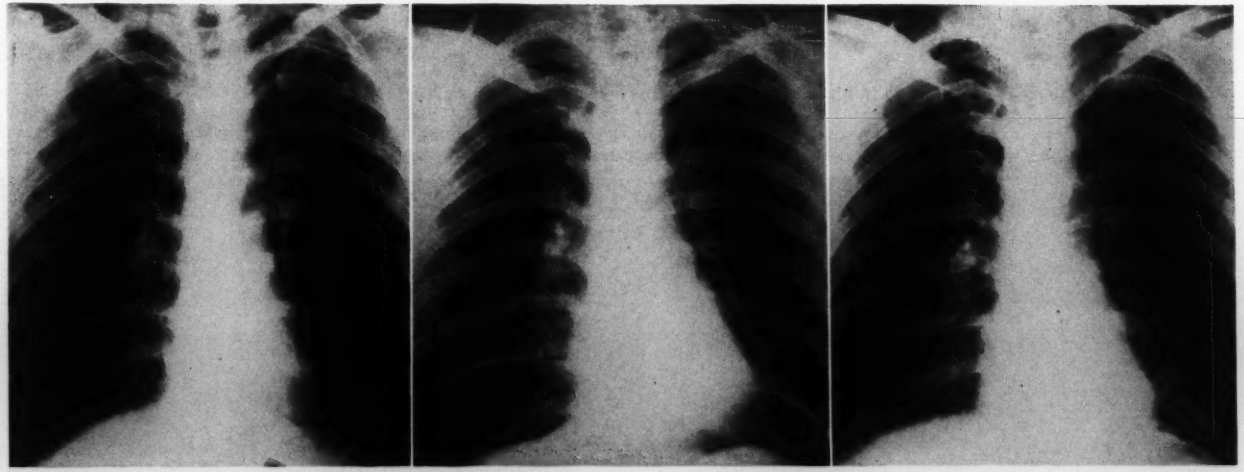

FIG. 1*A* (Left). Roentgenogram of July 20, 1954, showing a large cavity opposite the first intercostal space, in the left upper lobe. The ringlike shadow behind the first rib on the right side was subsequently thought to be due to bone structure as seen on tomograms.

FIG. 1*B* (Center). Chest film of June 6, 1955, showing an apparent disappearance of the cavity following therapy with antimicrobials and pneumoperitoneum.

FIG. 1*C* (Right). An outpatient follow-up chest film obtained on December 6, 1957, showing a fresh area of disease in the left cardiophrenic angle, probably the left lower lobe. In retrospect, this could have been a spread of histoplasmosis.

FIG. 1*D* (Left). Roentgenogram of January 7, 1959, showing definite reappearance of cavitation in the left upper lobe. Patient in hospital.
FIG. 1*E* (Center). Tomogram of January 7, 1959, confirming the presence of multilocular cavitation in the left upper lobe. The patient's sputum was positive for *H. capsulatum*.
FIG. 1*F* (Right). Chest film obtained August 5, 1959, following resectional surgery and amphotericin B therapy. An uneventful recovery followed.

evaluated by a U. S. Public Health Service cooperative study. It is hoped to find a drug easier to administer and less toxic than amphotericin B. The need for this is felt because of the increase in the number of cases being diagnosed. Another important factor to note is that at least 43 of 183 patients, mostly untreated and reported as having been followed up, have died of progressive histoplasmosis (2, 3).

In the present small series, pneumoperitoneum was tried as specific therapy but has been found ineffective. Several other cases of histoplasmosis with cavitation were treated with amphotericin B, but not with pneumoperitoneum. It is noteworthy that little change was observed in these cavities.

Five of the patients had cavities situated in the upper lobe and surrounded by dense pleural adhesions and fibrosis. Three of these cavities had open bronchi demonstrated by the injection of radiopaque material into the cavities. During this procedure, atmospheric pressures were recorded inside these three cavities so that the element of tension was nonexistent in these cases. Thus, the overlying fibrosis may explain the nonclosure of the cavities in these patients, but not in the others. Biologic factors as yet unexplained may also exist to account for nonclosure of mycotic cavities.

Sandler (7) suggested that the mechanism producing tension cavities in tuberculosis may also play a part in the mycoses. It is conceivable that tension cavities occur more frequently in pulmonary mycoses because of growth at the bronchocavitary junction, thus causing intermittent blockage, as in the case of aspergillosis. Tension cavities could well explain the poor response to collapse measures. On the other hand, Sweany and associates (8) have shown that parasites in histoplasmosis were rather scarce in the cavity walls and difficult to culture. They found the organisms in great numbers, however, in adjacent encapsulated caseous lesions.

There has always been considerable debate as to the mode of closure of tuberculous cavities under collapse therapy. Undoubtedly, some of the mechanical factors are common to tuberculous as well as to histoplasmal cavities; nevertheless, the latter do not appear to respond to treatment. In 1933, Coryllos (9) pointed out that the closure of tuberculous cavities depended upon closure of the draining bronchi. Eloesser (10) later emphasized the relationship of the cavity to the bronchial tree as being of clinical significance. Auerbach and Green (11) believed that occlusion at the bronchocavitary junction, usually involved in the disease process, was important for cavity closure. With the advent of chemotherapy, Auerbach believes the bronchocavitary junction often remains wide open and that the cavity closes by inspissation. Loesch (12) and Beatty (13) have also attached great significance to the state of the surrounding lung parenchyma, e.g., surrounding areas of fibrosis and adhesions tend to prevent cavity closure irrespective of the state of the bronchocavitary junction. Canetti and associates (14, 15) stated that, in the absence of chemotherapy, tuberculous cavities healed because the physiochemical environment was rendered unhealthy for the survival of tubercle bacilli. In treated resected lesions, viable bacilli were recovered more often from the open cavity than from closed necrotic foci. This is in contrast to the findings in histoplasmosis (8).

The cavity closure observed in the case described in detail was only apparent, and it is believed the cavity would not have reappeared so soon had it been tuberculous and had the patient received thirty-three months of chemotherapy and pneumoperitoneum. This suggests that closure of histoplasmal cavities should be checked by tomograms, as true cavity closure was not obtained in the present series. Due to limited experience with histoplasmosis, it is impossible to say with certainty whether pneumoperitoneum is more effective in tuberculous than in histoplasmal cavitary disease, although this appears to be the case. Indeed, if the physiochemical requirements of *H. capsulatum* are such that the organisms survive better in necrotic foci, then collapse procedures which block cavities would be contraindicated in some cases. Further morphologic, immunologic, and biochemical studies need to be carried out before answers to such questions will be available.

Summary

A typical case of pulmonary histoplasmosis is presented. Initially, this had been treated as pulmonary tuberculosis for a fairly long period on a presumptive diagnosis.

Six other cases are analyzed, some of these having been proved by the demonstration of the

presence of *H. capsulatum*. It appears from this small series that mycotic cavities do not respond to partial collapse measures such as pneumoperitoneum and, hence, tomography should be employed routinely before assuming cavity closure.

Sumario

Estudio de la Histoplasmosis Pulmonar con Referencia Particular al Neumoperitoneo Terapéutico

Preséntase un caso típico de histoplasmosis pulmonar, que al principio había sido tratado como tuberculosis pulmonar durante un período bastante largo de tiempo a base de un diagnóstico presuntivo.

Se analizan otros 6 casos, algunos de los cuales habían sido comprobados por una micología positiva. A juzgar por esta pequeña serie, parece que las cavernas micóticas no responden a las medidas parciales de colapso, tales como el neumoperitoneo. Hay que comprobar todos los casos con la tomografía antes de dar por sentado el cierre de las cavernas.

Resume

Une étude sur l'histoplasmose pulmonaire mentionnant spécialement son traitement par pneumopéritoine

Les auteurs présentent un cas typique d'histoplasmose pulmonaire. Ce cas fut traité initialement comme une tuberculose pulmonaire pendant une assez longue période, à la suite d'un diagnostic de présomption.

Six autres cas sont analysés parmi lasquels certains ont été prouvésê tre d'origine mycotique. Il semble d'après cette courte étude que les cavernes d'origine mycotique ne répondent pas à la collapsothérapie partielle, telle que le pneumopéritoine. Chaque cas devrait être vérifié par tomographie avant d'affirmer que la cavité est fermée.

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A RAPID MOUSE TEST FOR THE DIAGNOSIS OF PULMONARY TUBERCULOSIS[1,2]

III. Validation of the Test with Specimens from 1,000 Patients

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INTRODUCTION

One of the major problems in the treatment and control of tuberculosis is the long interval required to find out whether the patient is discharging viable tubercle bacilli. The usual cultural procedures require three to four weeks before sufficient growth of tubercle bacilli occurs for the culture to be reported positive and, generally, six to eight weeks before the culture is reported negative. In previous publications (1, 2) the writers described a mouse test which would give pertinent information to the clinician within twenty days. In a preliminary study of forty-six sputum specimens from sixteen patients previously known to have yielded cultures positive for tubercle bacilli (2), all sixteen patients produced at least one specimen positive by the mouse test in *five* days and by culture in *forty-six* days.

This report concerns the validation of the mouse test with 1,000 specimens routinely received in the laboratory to be studied for the presence of tubercle bacilli. This involved 4,400 mice, 11,000 slides, 12,000 cultures, and approximately 192,000 culture readings. The study was completed in sixteen months.

MATERIALS AND METHODS

The 5 per cent mucin suspension was prepared as described by Gale and Elberg (3) and in previous publications. Fresh mucin suspensions were made and used daily because aggregation of the granules occurred when the neutralized mucin suspensions were allowed to stand at 4°C., at room temperature, or at 37°C. for more than twenty-four hours. Such aggregated suspensions were difficult to manipulate and were not as effective in "virulence enhancement" as were fresh preparations. Careful attention should be given to the heat sterilization of the mucin suspension at an acid pH because the mucopolysaccharide components essential for "virulence enhancement" are heat labile at neutral or alkaline pH and are hydrolyzed with prolonged heat.

One hundred specimens per week (20 specimens per day), in alternating weeks, were tested by the mouse test and by culture and microscopy during the period January 13, 1958, to July 11, 1958. The specimens were digested and concentrated with 4 per cent sodium hydroxide plus 1 per cent alum. The neutralized sediments were suspended as homogeneously as possible in 1.5-ml. nutrient broth by the use of 1.0-ml. tuberculin syringes with 24-gauge needles. One milliliter was used for inoculation of mice, and the remaining 0.5 ml. for inoculation of two tubes of American Trudeau Society medium and for stained smear.[3] Before the mice were inoculated, 0.05 ml. of the sediment suspensions was pipetted into thioglycollate broth to check for contamination. The results of this contamination check have been reported elsewhere (4).

Four mice per sediment were inoculated intraperitoneally with 0.8 ml. of 5 per cent mucin suspension on one side of the abdomen, followed immediately by 0.2 ml. of sediment suspension on the other side of the abdomen.[4] One mouse in each group was sacrificed at five, ten, fifteen, and twenty days, respectively, with chloroform. The sacrificed mice were examined for gross pathology with particular reference to enlargement of the spleen. The spleen and/or any other organ showing lesions were removed and minced as thoroughly as possible. Two impression smears per spleen or organ were prepared for acid-fast staining. A small amount of minced spleen or organ was also used for culture on two tubes of American Trudeau Society medium. As much spleen surface as possible was impressed on the slides to minimize sampling errors.

The impression smears were fixed with methanol, flamed, and stained by the Ziehl-Neelson method on a staining rack on which the slides were separately placed, horizontally. Use of "cold" stains, such as the Kinyoun stain commonly

[1] From the Veterans Administration Hospital, Albuquerque, New Mexico.

[2] Presented in part before the Medical Session, as part of *Section 4B*, at the annual meeting of the American Trudeau Society, Chicago, Illinois, May 26, 1959.

[3] Acid-fast smears of gastric aspirates, or of sputum from outpatients, are not routinely done in this laboratory.

[4] It is believed that mixing of a viscous preparation such as mucin with an inhomogeneous material such as the sediment suspension will not ensure uniform aliquot inoculation. The peristaltic action of the intestines and the movement of the omentum mix the mucin and sediment inocula in the peritoneal cavity.

employed in histopathology, was not suitable because of poor fixation of material to the slide and the possibility of false positives by flotation of material from one slide to another in the staining container. The stained smears were examined for ten minutes before they were judged to be negative. All positive smears were verified by another examiner. A specimen was considered positive for tubercle bacilli by the mouse test if *two* criteria were met: acid-fast organisms in the impression smears of the spleen *and* enlargement of the spleen or lesions.

Swiss albino mice obtained from four sources were used: two strains from our own breeding colonies derived from Namru and Taconic Farms stock, and two strains from two commercial breeders. The 4 mice per sediment were kept in separate containers and fed laboratory chow (mouse pellets) and water ad libitum. There was no differentiation by sex or age, although mice older than five months were not used. It was the purpose of this validation study to use easily available mice which are more difficult to infect with tubercle bacilli than are pigmented mice on special diets. This technique could thus be used by any laboratory.

Deaths which occurred were not used in the assessment of results. Deaths[5] which took place forty-eight hours after inoculation were believed to be nonspecific, due to either toxic materials in the sediments, to enhancement of other organisms by the mucin, or to enhancement of toxic products liberated by tubercle bacilli in their *in vivo* milieu. The syringes and needles containing mucin were changed frequently to prevent carry-over of coliforms from one mouse to another.

Cultures were read twice weekly to determine positive results as quickly as possible. A culture was considered negative if no growth of tubercle bacilli occurred in eight weeks.

In order to preserve complete objectivity and freedom from bias in this validation study, the following procedure was used. A code number was assigned to each specimen as it was received. Such code identity was the only identification throughout the study. The senior investigator maintained a master record of the specimen code number and the identity of the patient. This record was not consulted until all of the data had been collected and correlated. Cultures were read and recorded by the identifying code number. Gross pathologic findings, smear examination results, and spleen culture results for each mouse were recorded on a special form which was devised with the code number as sole identification. A master sheet for each patient was prepared to include all of the information on the specimens pertaining to that patient after all the data on all of the specimens had been collected. The correlations and analyses described in this paper were done from these master sheets.

[5] Many of the mice received from one commercial breeder died in large numbers upon receipt, or within forty-eight hours after inoculation of sediment plus mucin. *Salmonella enteriditis* was isolated from the organs of the dead mice as from sacrificed stock animals. The use of such infected mice in this study did not seem to influence the results obtained beyond the greater number of deaths in the test animals.

TABLE 1

THE SOURCE OF PATIENTS AND SPECIMENS

Ward	Patients	Specimens
Tuberculosis wards	188	612
Nontuberculosis wards	55	113
Outpatient services	119	287
Total	362	1,012

RESULTS

A total of 1,012 specimens from 362 patients were used in this study (table 1). The group of 362 patients was made up of 188 (52 per cent) from tuberculosis treatment wards; 55 (15 per cent) from medical wards, and 119 (33 per cent) from outpatient services. It was anticipated that the 400 specimens from the 164 patients on medical wards and from outpatient services would serve as an internal control in the study. The types of specimens received (table 2) were: sputum, 50 per cent; gastric lavages, 46 per cent; and extrapulmonary secretions, 4 per cent.

Results obtained by mouse test and by culture: A comparison of the mouse test with culture results (tables 3 and 4) showed the following:

The presence of tubercle bacilli was established in the specimens from 74 patients (20 per cent) by both mouse test and culture. At least one specimen from 63 of these patients was positive by mouse inoculation within five days, and positive reactions were seen in 72 of these patients within ten days.

TABLE 2

THE TYPE OF SPECIMENS

Category	Total	In-patient	Out-patient
Sputum	508	418	90
Gastric lavages	462	279	183
Urine	30	16	14
Miscellaneous	12	12	
Total	1,012	725	287

Only one specimen from one patient (0.3 per cent) gave positive results by culture but was negative by the mouse test. This outpatient yielded the one specimen of five submitted which showed one colony resembling *Mycobacterium tuberculosis*. The negative mouse test may have been a sampling error.

In 64 patients (18 per cent), at least one specimen yielded evidence of tubercle bacilli by the mouse test, but was negative by both culture and smear examination. Specimens of approximately 51 per cent of these patients gave positive results by the mouse test in five days, specimens of 80 per cent in ten days, and specimens of 97 per cent within fifteen days.

Positive results by mouse inoculation were found in specimens of 5 patients (1.4 per cent) which were contaminated by culture.

The specimens of the remaining 218 patients (60 per cent) were negative by both techniques.

Essentially similar results were obtained with regard to specimens: 159 specimens (16 per cent) were positive for tubercle bacilli by both mouse test and culture, 3 specimens (0.3 per cent) were positive by culture but negative by mouse test,

TABLE 3

COMPARISON OF RESULTS OBTAINED BY CULTURE AND BY MOUSE TEST, FOR THE PRESENCE OF TUBERCLE BACILLI (362 PATIENTS*)

Category	Total		Tuberculosis Wards	Nontuberculosis Wards	Outpatient
	Number	Per Cent			
Positive culture; positive mouse test	74	20.4	65	5	4
in 5 days	63	17.4	54	5	4
10 days	9	2.5	9		
15 days	2	0.5	2		
Positive culture; negative mouse test	1	0.3			1
Negative by both culture and microscopy; positive mouse test	64	17.7	43	7	14
in 5 days	33	9.1	22	4	7
10 days	18	5.0	11	2	5
15 days	11	3.0	9		2
20 days	2	0.6	1	1	
Contaminated culture; positive mouse test	5	1.4	3		2
Negative culture; negative mouse test	218	60.2	77	43	98

* A patient was considered "positive" if at least one specimen was positive for tubercle bacilli.

TABLE 4

COMPARISON OF RESULTS OBTAINED BY CULTURE AND BY MOUSE TEST FOR THE PRESENCE OF TUBERCLE BACILLI (1,012 SPECIMENS)

Category	Total		Tuberculosis Wards	Nontuberculosis Wards	Outpatient
	Number	Per Cent			
Positive culture; positive mouse test	159	15.7	149	6	4
in 5 days	135	13.3	126	5	4
10 days	20	2.0	19	1	
15 days	3	0.3	3		
20 days	1	0.1	1		
Positive culture; negative mouse test	3	0.3		1	2
Negative by both microscopy and culture; positive mouse test	99	9.8	66	11	22
in 5 days	45	4.4	32	6	7
10 days	29	2.9	17	2	10
15 days	23	2.3	16	2	5
20 days	2	0.2	1	1	
Contaminated culture; positive mouse test	18	1.8	15		3
Negative culture; negative mouse test	733	72.4	382	95	256

99 specimens (10 per cent) were positive by mouse test but negative by culture and smear examination, 18 specimens (2 per cent) were positive by mouse test but contaminated culturally, and the remaining 733 specimens (72 per cent) were negative by both techniques.

Rapidity of the mouse test as compared with culture: All 74 patients (100 per cent) were found to yield positive results in at least one specimen by mouse test in *fifteen* days and by culture in *fifty-two* days (figure 1). At ten days, none of the cultures were positive, while specimens from 72 patients (97 per cent) yielded positive results by mouse test. At fifteen days, 158 specimens (99.4 per cent) were positive by mouse test, whereas only 45 specimens (28 per cent) were positive by culture. These data confirm the previously reported observation (2) that the mouse test is more rapid than culturing procedures in demonstrating the presence of viable tubercle bacilli.

The 74 patients, some of whose specimens were positive by both mouse test and culture, furnished a total of 204 specimens. Of these,

159 specimens gave positive results by both techniques, 10 were positive by mouse test but negative by culture, 5 were positive by mouse test but contaminated culturally, 2 were negative by mouse test but positive by culture, and 28 specimens were negative by both mouse test and culture.

Comparison of results obtained by mouse test with those obtained by examination of stained smear: An essential part of the mouse test is the examination of spleen impression smears for acid-fast bacilli. It is possible that microscopic examination of the concentrates for the presence of acid-fast bacilli might yield the same information in less time than that required by the mouse test. The results of microscopy of sputum concentrates compared with those obtained by mouse test are shown in table 5. Only 34 of the 143 patients (23 per cent) and 97 of the 276 specimens positive by mouse test were also positive by microscopy (35 per cent). These represented patients consistently positive by culture who always excreted large numbers of tubercle bacilli in every specimen. It is evident that multiplication of tubercle bacilli occurs relatively rapidly in the animal body, since detectable acid-fast organisms were seen in spleen impression smears from 78 specimens that were negative on microscopy, and from 101 specimens not examined microscopically because of the recognized difficulty in finding acid-fast bacilli in smears of concentrates of gastric lavages, urine, et cetera. Of these 179 specimens, 96 (54 per cent) were positive for tubercle bacilli by mouse test within five days.

Question of "false positives" in the results obtained by mouse test: The finding that 64 patients yielded specimens positive by mouse test but negative by culture suggested two alternate possibilities: either the mouse test is more sensitive than culture or microscopy for demonstrating the presence of tubercle bacilli, or the mouse test produces many false positives and, therefore, would not be usable. It seemed likely that useful evidence to resolve this question would be found in the clinical data. Accordingly, the clinical charts and roentgenographic reports of the 362 patients in the study were carefully reviewed for clinical diagnosis, history, and status of disease at the time the specimens were sent to the laboratory. The bacteriologic findings on the 362 patients for the previous five years were also carefully checked, with particular reference to previous cultures positive for tubercle bacilli, and the ward physicians were consulted with regard to the current clinical status of these patients.

TABLE 5

COMPARISON OF RESULTS OBTAINED BY MICROSCOPY AND BY MOUSE TEST FOR THE DETECTION OF TUBERCLE BACILLI

Category	362 Patients		1,012 Specimens	
	Number	Per Cent	Number	Per Cent
Positive on microscopy; positive mouse test	34	9.4	97	9.6
Negative on microscopy; positive mouse test	46	12.7	78	7.7
Microscopy not done; positive mouse test	63	17.4	101	10.0
Positive on microscopy; negative mouse test	0	0	1	0.1

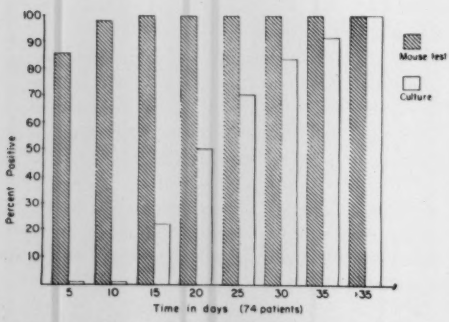

FIG. 1. Comparison in the time for mouse test and culture to be positive for tubercle bacilli.

The examination of the bacteriologic and clinical histories of the 64 patients (tables 6, 7; figure 2) strongly suggested that, rather than being productive of false positive reactions, the mouse test is more sensitive than culture techniques in the detection of tubercle bacilli. Of the 64 patients concerned, 54 had previous cultures positive for tubercle bacilli. In 20 patients, a culture had been positive within six months, in 31 patients it had been positive within twelve months, and in 42 patients a culture had been positive within eighteen months. Ten patients[6]

[6] The 10 patients gave the following histories: one patient had been found at autopsy to have had

TABLE 6

THE CORRELATION OF PATIENTS WITH SPECIMENS POSITIVE BY MOUSE TEST BUT NEGATIVE BY CULTURE, WITH PREVIOUS BACTERIOLOGIC HISTORY

Category	Patients			
	Per Category		Cumulative	
	Number	Per Cent	Number	Per Cent
Negative by culture and microscopy; positive mouse test	64	100	64	100
Last positive culture, previously, by months				
3	6	9.4	6	9.4
6	14	21.8	20	31.2
9	3	4.7	23	35.9
12	8	12.5	31	48.4
18	10	15.6	41	64.0
24	1	1.6	42	65.5
>24	12	18.7	54	84.3
No previous positive culture	10	15.6	—	—

had had no previous positive cultures, although their clinical histories revealed either existing tuberculosis or good presumptive evidence for tuberculosis (table 6). Significantly, 10 patients in this group showed positive cultures subsequently. Clinically, 26 patients had diagnoses of active tuberculosis, 27 patients had inactive disease; and 5 patients had tuberculosis in areas other than the lung (table 7). Of the 12 patients with far advanced active disease, all had cavities but yielded no tubercle bacilli on culture. Six patients were not diagnosed as tuberculous, but had histories suggestive of tuberculosis, either because of findings of recent single positive cultures with no clinical or roentgenographic evidence of the focus of infection, as with 3 patients,[7] or because of compatible history.[8]

TABLE 7

THE CORRELATION OF PATIENTS WITH SPECIMENS POSITIVE FOR TUBERCLE BACILLI BY MOUSE TEST BUT NEGATIVE BY CULTURE, WITH CLINICAL DIAGNOSIS*

Category	Patients	
	Number	Per Cent
Negative by culture and by microscopy; positive mouse test	64	100
Tuberculosis:		
Active:	26	40.6
Far advanced	12	18.7
Moderately advanced	11	17.2
Minimal	3	4.7
Inactive:	27	42.2
Far advanced	11	17.2
Moderately advanced	6	9.4
Minimal	10	15.6
No tuberculosis	6	9.4
Other tuberculosis	5	7.8

* In accordance with the *Diagnostic Standards and Classification of Tuberculosis* of the National Tuberculosis Association (5).

The correlation of both clinical diagnosis and previous positive cultures for the 64 patients (figure 2) also demonstrates the strong improbability that the mouse test is productive of false positives. A large number of patients in this group had clinically active tuberculosis and had had cultures positive for tubercle bacilli within the preceding eighteen months. Such patients

miliary tuberculosis, but it had not been diagnosed prior to death; 2 patients had tubercles in resected lung specimens as shown by a pathology report; one patient had pleural effusion; 2 patients showed clinical and roentgenographic evidence of pulmonary tuberculosis, but whether the disease was active had not been determined; one patient had proved tuberculosis since 1919, and hemoptysis and hemothorax in 1930; one Indian patient with a diagnosis of chronic glomerulonephritis furnished a urine specimen which was positive by mouse test but negative for tubercle bacilli on culture; one patient with lung abscess showed acid-fast bacilli on direct smear of sputum taken on admission prior to chemotherapy, but was positive for tubercle bacilli by mouse test and negative by culture on two sputum specimens four months after the start of therapy; and one patient had hemoptysis and weight loss on admission and no roentgenographic evidence of pulmonary disease. However, his wife had far advanced tuberculosis, one child had advanced tuberculosis and was in a sanatorium, and a brother who had lived with the family had died of tuberculosis a year before. (The latter 3 diagnoses were in accordance with the *Diagnostic Standards* of the National Tuberculosis Association.)

[7] One of these patients with bronchogenic carcinoma was shown on autopsy to have had bilateral cavitation and evidence of extensive tuberculous infection.

[8] The latter 3 patients described in footnote 6.

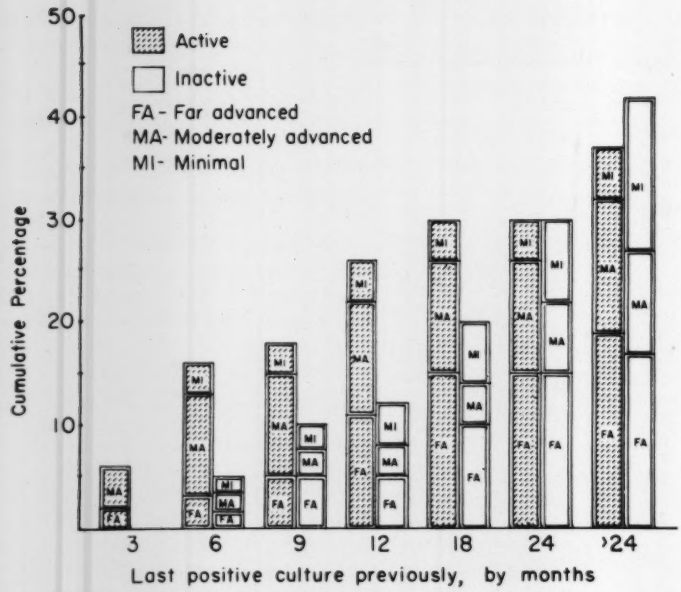

FIG. 2 The relationship of the clinical diagnosis and history of previous cultures positive for tubercle bacilli among patients positive by mouse test but negative by culture.

could be expected to excrete tubercle bacilli at some time.

Further evidence of the greater sensitivity of the mouse test is found in the examination of culture results: Positive cultures with 15 colonies or less were found in 40 specimens from 31 patients who yielded a total of 52 specimens positive by culture and 71 specimens positive by mouse test.

A comparison of the results obtained by culture, by mouse test, and by splenic culture of the injected mice is shown in table 8. Of the 276 specimens positive by mouse test, 157 specimens (57 per cent) were negative or contaminated in cultures of the spleens of these mice. Only 44 specimens (28 per cent) were negative or contaminated in splenic cultures of the mice injected with the 162 specimens that were also positive for tubercle bacilli by culture.

Deaths of the inoculated mice: Deaths within forty-eight hours were not used in assessment of the data but were considered to be nonspecific. Specimens which produced death in all 4 of the mice injected were withdrawn from the sampling, since comparison of the results of culture and the mouse test was not possible. There were 25 such specimens. The relationship of specimens positive for tubercle bacilli by mouse test and the number of deaths per specimen appears in table 9. It is of interest that positive mouse test results were found in approximately one-third of the speci-

TABLE 8

CORRELATION OF RESULTS OBTAINED BY CULTURE, BY MOUSE TEST, AND BY MOUSE SPLENIC CULTURE

Culture	Mouse Test	Mouse Splenic Culture	1,012 Specimens	
			Number	Per Cent
+	+	+	115	11.3
+	+	0	44	4.3
+	0	0	3	0.3
+	0	+	0	0.0
0	0	+	1	0.1
0	0	0	732	72.3
0	+	0	113	11.1
0	+	+	4	0.4
Total	+	+	119	43.0
	+	0	157	56.6
	0	+	1	0.4

+ = positive.
0 = negative.

TABLE 9

THE RELATIONSHIP OF DEATHS OF MICE TO MOUSE TESTS POSITIVE FOR TUBERCLE BACILLI

Category	Total	Specimens					
		Positive		Positive by:			
		Number	Per Cent	5 days	10 days	15 days	20 days
Positive culture: positive mouse test	1,012	159	15.7	135	20	3	1
0 deaths	393	82	21.1	73	8		1
1 death	333	50	15.0	38	9	3	
2 deaths	190	18	9.5	15	3		
3 deaths	96	9	9.4	9			
Negative culture; positive mouse test	1,012	117	11.6	52	34	29	2
0 deaths	393	47	12.0	19	10	16	2
1 death	333	42	12.6	15	14	13	
2 deaths	190	25	13.2	15	10		
3 deaths	96	3	3.1	3			
Total positive mouse tests	1,012	276	27.3	189	52	32	3
0 deaths	393	129	32.5	94	16	16	3
1 death	333	92	27.5	53	23	16	
2 deaths	190	43	22.6	30	13		
3 deaths	96	12	12.5	12			

mens producing no deaths, one-quarter of the specimens yielding one or 2 deaths, and one-eighth of the specimens with 3 deaths. With specimens positive by both mouse test and culture, approximately the same percentage of positive mouse tests was obtained, regardless of whether there were one, 2, or 3 deaths (15.0 per cent, 9.5 per cent, and 9.4 per cent, respectively). There was also little difference in the incidence of positive mouse tests between specimens producing zero, one, or 2 deaths (12.0 per cent, 12.6 per cent, and 13.2 per cent, respectively) in the category of specimens which were positive on mouse test but negative on culture.

Control Studies

Control experiments were set up to answer the following questions: (*1*) Could cross infection occur from one cage to another (as has been shown to occur with tuberculous guinea pigs) or with mice inoculated with tubercle bacilli by the intravenous or intranasal routes (6)? (*2*) Could cross infection occur in mucin-treated mice, since all of the tested specimens were inoculated intraperitoneally with mucin? (*3*) Could false positive results be obtained by manipulative procedures in autopsy, preparation of impression smears, staining of the smears, or other techniques employed?

The following experiment was done to answer the question of cross infection from one cage to another:

One hundred and twenty mice (60 each from the two breeding colonies) were set in cages of 4 mice each, in close proximity to the cages of test mice on every shelf of the racks holding the cages as well as at various places in the infected rooms.[9] One mouse in each of these control cages was sacrificed daily. Autopsies were performed and impression smears and cultures of the spleens were made for detection of acid-fast bacilli. In this way, noninoculated, normal mice were exposed to contact with the possibly contaminated atmosphere of the infected rooms[9] from one day to sixty days. *None of these mice were found to yield acid-fast bacilli on either smear examination or culture.*

The second control experiment was planned to answer the question of cross infection to mucin-injected control mice:

Four mice per group were injected with mucin and saline each week in which specimens were inoculated. These mice were sacrificed, examined

[9] Ultraviolet lights are placed high on the wall in these enclosed rooms, and are lighted constantly when personnel are not in the rooms.

at autopsy, and impression smears and cultures were prepared at five-day intervals in the same manner as for the test animals. In addition, 30 mucin-injected mice were placed similarly to the normal controls, described above, were sacrificed at two-day intervals, and examined similarly. *None of the mucin-treated mice were found to yield acid-fast bacilli on either smear examination or culture.*

In performing the autopsies, the following procedures were used: The sacrificed mouse was placed on the autopsy board (7) with its ventral surface up. The scissors and forceps were dipped into a container of 95 per cent ethanol, set aflame, allowed to cool, and dipped again and flamed, a total of five times. An incision was made over the abdomen and thorax, and the skin was dissected free and peeled away. The instruments were dipped in alcohol and flamed five times. After cooling, the peritoneal wall was opened and peeled back. The instruments were again dipped in alcohol and flamed five times, and examination of liver, spleen, omentum, mesentery, kidney, and other tissues was performed. When an organ was to be removed, the instruments were again flamed five times and used until the next organ was to be removed. The instruments were thus continuously sterilized during the course of the autopsy. When the autopsy was completed, the instruments were flamed, and tissue debris was scraped free with a scalpel. The instruments were then flamed five times and the spleen was minced for preparation of impression smears and culture. When another organ was to be minced for smear and culture, the instruments were flamed, cleaned, flamed again five times, and were ready for the next autopsy. In this manner, four to five pairs of sharp instruments could be used for the twenty to forty autopsies performed daily.

It is conceivable that acid-fast organisms could be carried over from the organs of one infected mouse to the succeeding noninfected animal and, thus, produce false positive results.

TABLE 10

THE DECONTAMINATION OF INSTRUMENTS BY FLAMING IN ALCOHOL

Technique	Scissors			Forceps		
	Culture for Tubercle Bacilli*		Smear†	Culture*		Smear†
	21 days	8 weeks		21 days	8 weeks	
1. Emulsion	3+	4+	Very many	3+	4+	Very many
Times flamed:						
1	0	0	Many	0	0	Many
2	0	0	Few	0	0	Few
3	0	0	Rare	0	0	Rare
4	0	0	Rare	0	0	Rare
5	0	0	Neg.‡	0	0	Neg.‡
2. Infected spleen	2+	4+	Very many	2+	4+	Very many
Times flamed:						
1	0	0	Rare	0	0	Rare
2	0	0	Neg.‡	0	0	Neg.‡
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0
3. Infected, cleaned, flamed 5 times → spleen	0	0	0	0	0	0
Spleen	0	0	0			

* Culture:
0: no growth or negative
3+: greater than 200 colonies, but not confluent
4+: confluent growth

† Smear:
Very many—more than 20 organisms per oil-immersion field
Many—10–20 organisms per oil-immersion field
Few—1–5 organisms per oil-immersion field
Rare—1–5 organisms per slide

‡ Neg.—acid-fast debris seen, but no organisms

This was tested in the following experiments performed on two different occasions:

(*1*) An emulsion of H37Rv was prepared to provide approximately 10^7 viable units per milliliter of broth. Sterile scissors and forceps were dipped into the emulsion. The cutting edges of the scissors and the toothed surfaces of the forceps were swabbed with broth-moistened cotton-tipped applicators. Two tubes of American Trudeau Society medium and one slide for acid-fast staining were inoculated from each instrument. The instruments were dipped into the emulsion, then into ethanol, and set aflame, once. When the flames had subsided and the instruments were cool, they were swabbed, cultured, and smeared. The instruments were dipped into the emulsion, dipped in alcohol and flamed twice, and swabbed, cultured, and smeared. This was repeated until the instruments had been contaminated and flamed three, four, and five times, respectively.

(*2*) Eight normal mice were sacrificed and their spleens removed. Sterile scissors and forceps were dipped into the H37Rv emulsion and used to mince one spleen finely. As above, the instruments were swabbed, cultured, and smeared. Similarly, the instruments were dipped into the emulsion and used to mince a spleen. They were flamed, once, twice, three, four, and five times, respectively, and swabbed, cultured, and smeared.

(*3*) A spleen from a normal mouse was suspended in the H37Rv emulsion for five minutes. Sterile scissors and forceps were dipped into the emulsion and used to mince the infected spleen. The instruments were cleaned free of tissue debris and flamed five times. These instruments were then used to mince a normal spleen. The instruments were swabbed, cultured, and smeared. The normal spleen mince was also cultured and smeared.

The conclusions (table 10) were: (*a*) One alcohol flaming of contaminated instruments renders the organisms negative by culture, but still apparent by smear examination. (*b*) A minimum of five flamings is required before the organisms are no longer visible by smear examination, and positively negative by culture.

Discussion

These data indicate both the rapidity and the greater sensitivity of the mouse test as compared with conventional culture and smear procedures. Several questions may arise: (*1*) Why should the mouse test be more sensitive than the usual culture techniques? (*2*) What are the clinical implications of a positive mouse test for tubercle bacilli when culture results have been negative for a long time? (*3*) Is the patient whose specimens have yielded a positive mouse test and negative cultural results a public health problem, or a possible reinfection problem to himself? (*4*) Can the mouse test also detect the unclassified mycobacteria?

Possible reasons for the greater sensitivity of the mouse test as compared with culture techniques may be: (*a*) trace amounts of chemotherapeutic agents in the specimen may affect culture results adversely, but would be less likely to affect the situation in the mouse; (*b*) the organisms might be altered through treatment received by the patient (for example, isoniazid), so that their viability in culture medium might be at low ebb while ability to survive *in vivo* might be less affected; (*c*) trace amounts of broad-spectrum (tetracycline) mashes may be present in the eggs used for culture media, so that only large numbers of organisms may grow on the culture medium, whereas relatively few tubercle bacilli can multiply in the mouse; (*d*) concentration procedures render large numbers of tubercle bacilli poorly viable so that only one colony or no growth at all may occur on artificial culture medium, whereas the mammalian body can provide proper oxygen tension, carbon dioxide, pH, or other possible factors to enhance the growth of such poorly viable organisms; (*e*) culture media may become contaminated with other organisms not destroyed in concentration procedures (4) and, thus, not support the growth of tubercle bacilli (the mouse can handle such contaminants with little adverse effect); (*f*) tubercle bacilli can probably multiply more quickly in the mammalian body than in artificial culture medium.

The questions concerning the significance of a positive mouse test and negative cultures in terms of the public health, reinfection, or importance to the clinician in his therapy are not easily answered. It is believed that small numbers of tubercle bacilli are being excreted by such patients, probably from a small focus of infection, but not in sufficient amount to be highly communicable. However, if the local resistance of the patient breaks down, as with concomitant respiratory infection, trauma, or other stress, many more organisms may be excreted to make the patient much more infectious to others or a

hazard to himself. These patients should be followed very closely, and specimens taken very frequently. As long as the patient is excreting sufficient organisms to make the mouse test positive, chemotherapy should be continued until the mouse test results become negative.

The ability of the mouse test to detect unclassified mycobacteria could not be determined in this study because only one of the 1,016 specimens sampled yielded a scotochromogenic culture, and only 2 specimens from one patient produced photochromogenic cultures. The mouse test results of the specimen yielding the scotochromogenic culture showed the atypical morphology associated with these forms: the long, broad organism with faintly acid-fast cytoplasm and many darkly acid-fast granules, giving a beaded or barred effect. The mouse test results of the photochromogen-yielding specimens showed morphologically "typical" tubercle bacilli, similar to those obtained from the nonpigmented colony prior to exposure to light (8). However, there were 27 patients in this study whose last positive cultures had yielded unclassified mycobacteria of the scotochromogenic form. Of these 27 patients, 7 produced specimens which were positive by the mouse test but negative by culture. The specimens from the other 20 patients were negative by both techniques. The mouse test results of specimens from 4 of these 7 patients showed the atypical morphology mentioned above.

Summary

A mouse test to detect tubercle bacilli in sputum and other bodily discharges has been validated with 1,016 specimens routinely received in the laboratory. The mouse test was not only more rapid, but was also more sensitive than usual culture procedures for the detection of tubercle bacilli. Of the 362 patients in the study, 145 patients (40 per cent) yielded specimens which were positive by the mouse test in twenty days, whereas the specimens from 75 patients (20 per cent) were positive by culture in fifty-two days. Of the 75 patients whose specimens were positive by culture, 84 per cent gave positive results by mouse test in *five* days and 96 per cent in *ten* days, whereas *no* cultures were positive in ten days.

Sumario

Rápida Prueba del Ratón para el Diagnóstico de la Tuberculosis Pulmonar. III. Validación de la Prueba en Ejemplares de Mil Enfermos

Una prueba del ratón dedicada a descubrir bacilos tuberculosos en ejemplares de los enfermos ha sido validada en 1,017 ejemplares de los recibidos corrientemente en el laboratorio. La prueba del ratón resultó no tan sólo más rápida sino también más sensible que los habituales procedimientos de cultivo. De los 362 enfermos del estudio, 143 (40 por ciento) tuvieron ejemplares que fueron positivos con la prueba del ratón en veinte días en tanto que los ejemplares de 75 enfermos (20 por ciento) resultaron positivos al cultivo en cincuenta y dos días. De los 75 que fueron positivos al cultivo, 84 por ciento dieron resultados positivos con la prueba del ratón en *cinco* días y 96 por ciento en *diez* días, mientras que ningún cultivo resultó positivo en diez días.

Resume

Un examen rapide sur la souris pour diagnostiquer une tuberculose pulmonaire. III. Valeur de l'examen avec l'expectoration de 1000 malades

On a estimé la valeur d'un examen pratiqué sur la souris pour détecter des bacilles tuberculeux dans l'expectoration de 1017 malades, l'expectoration étant envoyée systématiquement au laboratoire. L'examen sur la souris ne fut pas seulement plus rapide mais fut aussi plus sensible que les procédés habituels de culture. Parmi les 362 malades étudiés, 143 (40%) eurent des expectorations positives avec cet examen, dans les vingt jours, alors que l'expectoration de 75 malades (20%) fut positive en cinquante deux jours avec la méthode de culture. Parmi les 75 malades dont l'expectoration eut une culture positive, 84% eurent un résultat positif en 5 jours avec le test de la souris et 96% en 10 jours, alors qu'aucune culture ne fut positive en 10 jours.

Acknowledgments

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Case Report

PERIPHERAL NEURITIS DUE TO STREPTOMYCIN[1]

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Introduction

Streptomycin plays an important role in the current treatment of tuberculosis. Various side effects and signs of toxicity have been reported since this drug came into use. Vertigo, nausea, and ataxia as a result of involvement of the vestibular apparatus are the most usual. Circumoral and other transient paresthesias of the face on the days of streptomycin administration are well known. In an occasional patient there may be hypersensitivity reactions, often arising within two weeks after the start of treatment and evidenced by fever, rarely urticaria, more often skin rashes usually of the maculopapular type. Streptomycin is also one of the many drugs that may cause an eosinophilia in the peripheral blood, usually without any other hematologic or allergic manifestations. Much more rare are true peripheral neuritides in the extremities; or at least they often may not be recognized as being due to streptomycin. A prominent physician in the field of tuberculosis stated that the following case was only the second he had ever seen (1). Also, the different manufacturers of the drug were not familiar with this possible complication (2).

Case Report

A 76-year-old white widow was admitted to the hospital on November 16, 1957, with far advanced bilateral cavitary pulmonary tuberculosis. The sputum was positive for acid-fast bacilli on microscopy. Peripheral blood examination was essentially within normal limits. Three weeks after treatment was started with 1 gm. of streptomycin three times weekly, 12 gm. of PAS, and 300 mg. of isoniazid daily in four divided doses, she developed symptoms of "PAS toxicity" in the form of gastrointestinal intolerance (anorexia, nausea, and vomiting). Various preparations of this drug in various doses were tried without success, and it was therefore discontinued one month later. Concentrations of PAS in the serum at the height of the toxic symptoms, however, were not higher than 2.5 mg. per 100 ml. of serum at four hours after the full breakfast dose of 3 gm. of PAS. Hence, it seemed most likely that this was not a "regular" intolerance, since in those cases the serum PAS concentration shortly before the noon dose is almost always more than 9 mg. per 100 ml. and often ranges from 15 to 25 mg. per 100 ml.

Streptomycin and isoniazid were continued and sputum cultures were positive for tubercle bacilli for the last time in January, 1958. On February 23, the patient complained of pain in the left hand. This became worse and kept her awake. In spite of high doses of pyridoxine, the pain worsened and isoniazid was discontinued while pyridoxine was kept up in three 50-mg. doses daily. The total concentration of isoniazid in the serum was 0.4 mg. per 100 ml. at four hours after the breakfast dose, with a free isoniazid concentration of 0.3 mg. per 100 ml. Total leukocyte and differential counts were essentially within normal limits. Meanwhile there was no clinical improvement of the patient's pain and, on May 4, there were the additional findings of hyperalgesia, hypo- and paresthesias of the dorsum of the left hand and wrist, together with motor neuron involvement (loss of grip) and marked nonpitting edema in the same area. These changes progressed to the point at which the patient had to be fed because she could not hold her fork anymore. The only drugs administered at that time were 1 gm. of streptomycin three times weekly, pyridoxine, and analgesics for the continuing severe pain.

On May 8, the concentration of streptomycin in the blood was found to be 2.1 mg. per 100 ml. at five hours after a 1-gm. dose intramuscularly, a high normal value. The total leukocyte count was 11,100 with 31 per cent eosinophils! Findings at four-day intervals were total leukocyte count of 8,900 with 28 per cent and 9,900 with 38 per cent eosinophils. On account of this eosinophilia, streptomycin was discontinued on May 20, and one week later the eosinophils had come down to 5 per cent.

Clinically, the pain in the hands improved and after one month the edema had disappeared, but there remained severe loss of motor function. Since then, after one year, the disability has gradually cleared completely on continuous exercise. The total leukocyte count has ranged

[1] From the Homer Folks Tuberculosis Hospital, Oneonta, New York.

from 6,500 to 8,800 with 2 to 6 per cent eosinophils.

On August 10, the patient had a "cold," with a productive cough. Sputum which had been negative for tubercle bacilli since February, 1958, was again positive, and roentgenograms of the chest showed dissemination of the disease that had become almost stable previously. The need for drug treatment was apparent and desensitization with Rezipas® (12 mg. four times a day on the first day and a double dose every other day) was successfully carried out. By September 18, 1958, the patient was receiving 5 gm. four times daily, or the equivalent of a total of 10 gm. of PAS daily. Streptomycin desensitization was then attempted, starting with 50-mg. doses and also doubling the dose every other day. Thus, after September 7, 1958, the patient received 1 gm. of streptomycin three times weekly. Two weeks later, on September 22, 1958, the same signs and symptoms as before recurred, which were typical of a true peripheral neuritis with sensory, motor, and autonomic involvement, and there was an eosinophilia in the peripheral blood of 16 per cent with a total leukocyte count of 10,700. On September 26, 1958, these values were 10 per cent and 9,000. On that same day dihydrostreptomycin was substituted for streptomycin; this drug was (and even now after ten months still is) well tolerated without any ill effects. On October 7, 1958, the total leukocyte count was 8,600 with 5 per cent eosinophils. Since then eosinophils have never been more than 4 per cent. Between November 3 and November 17, isoniazid was carefully and gradually added to the drug regimen, up to 250 mg. daily. Sputum became negative for tubercle bacilli on microscopy in April, 1959, but cultures had failed to grow since January, 1959.

Discussion

The fact that typical symptoms of a peripheral neuritis disappeared upon discontinuance of streptomycin and, after a free, symptomless interval, reappeared upon streptomycin administration is, in the opinion of the writer, sufficient proof of the genesis of the peripheral neuritis in this patient. It is of interest that the dihydro derivative of streptomycin failed to call forth the neuritic reaction; but there are many possibilities, such as allergic edema of the axon, eosinophilic infiltration of the nerve sheath, allergic changes and hyperpermeability of the vasa nervorum, leading to nutritional changes of the nerve, et cetera. The presumption might be that this neuritis occurred in a setting of serum sickness, as is seen particularly in neuritis due to tetanus antitoxin (3). This is particularly the case in view of the fact that dihydrostreptomycin fails to call forth the reaction produced by the streptomycin—a sequence of events common in hypersensitivity reactions to the latter drug. Whether the eosinophilia is to be regarded as an allergic manifestation to streptomycin (and not to dihydrostreptomycin) cannot be stated with assurance.

In the present case, the eosinophilia indeed disappeared when streptomycin was discontinued and reappeared after the drug was restarted; it is possible that this represented a manifestation of generalized hypersensitivity or even mild serum sickness. The fact that in the present patient about twelve months were needed for a *restitutio ad integrum* leads to the belief that there was not only edema of the axon, or only temporary changes in the vasa nervorum, but also actual nerve tissue involvement in possibly the same fashion as is seen in peripheral neuritis due to isoniazid or methyl alcohol (4, 5). It is impossible, however, to prove this point without microscopic examination of the nerve tissue in question, and this impression is based merely on clinical observation.

Summary

A case of peripheral neuritis due to streptomycin is presented. In cases of peripheral neuritis in patients receiving treatment for tuberculosis, isoniazid is the drug almost certainly blamed, as happened in the present case. The great danger is that the patient who manifests neuritis is considered without further investigation to be "isoniazid-hypersensitive," and the most important and powerful drug is permanently discontinued. In order to avoid this, it is of paramount importance to recognize the real culprit, which may be streptomycin in probably more instances than is realized. A trial with dihydrostreptomycin should be carried out in those cases.

Sumario

Neuritis Periférica Debida a la Estreptomicina: Presentación de un Caso

Se presenta un caso de neuritis periférica debida a la estreptomicina. En los casos de neuritis periférica en enfermos que reciben tratamiento por tuberculosis pulmonar, la isoniacida es la droga casi seguramente culpada, y así sucedió en el caso actual. El gran peligro es que, al enfermo que manifieste neuritis, se le considere sin más investigación como "isoniacido-hipersensible" y

se abandone permanentemente la droga más importante y poderosa. A fin de evitar esto, reviste importancia suprema el reconocimiento del verdadero culpable, que puede ser la estreptomicina probablemente en más casos de lo que se piensa. En esos casos, debe llevarse a cabo una prueba con la dihidroestreptomicina.

Resume

Nevrite périphérique due à la streptomycine. Rapport sur un cas

Un cas de névrite périphérique due à la streptomycine est presenté par l'auteur. Dans des cas de névrite périphérique chez des malades traités pour tuberculose, l'isoniazide est le médicament qui est le plus blamé comme ce le fut dans le cas rapporté. Le danger important est que le malade qui présente des signes de névrite est considéré, sans plus ample investigation, comme un sujet "hypersensible" à l'isoniazide et le plus important et le plus puissant médicament est interrompu complètement. Dans le but d'éviter ceci, il est d'une importance primordiale de reconnaître le vrai responsable qui peut être la streptomycine plus souvent qu'on ne le réalise. Une épreuve à la dihydrostreptomycine devrait être pratiquée dans ces cas.

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Editorial

The Community's Stake in Medical Research[1]

When barriers exist between the scientists and the community in which they live, the directions science takes are not always wise. For science is a form of creativity—in a sense, it is the major art form of our times. And, like the other creative art forms, science is never so robust as when it is reflecting the tribulations and the triumphs of its day. Could it not be that this is the real point we are missing in our new game of keeping up with the Soviet Joneses?

We were incredulous, awed, irritated, and somewhat scared by the success of the first Sputnik. We were then highly amused and a touch self-righteous at the spectacle of the futile attempts of the Soviet dictatorship to suppress another of man's creations, that by Boris Pasternak. But in our great compassion for Pasternak in his struggle against governmental tyranny, let us not forget that he too, just like the Soviet physicists, has devoted himself to the problems of his times. In short, Russian citizens won not just one Nobel prize in 1958 but *two*: one in the arts and one in the sciences, today's two major forms of creativity.

In other words, however unappealing their larger community might seem to us, the Soviet scientists are a part of it. In the history of the Western World, there have been times when the creative people were a true part of the larger community and times when they were really not.[2] This has been as true of the medical scientists as of the others, even though few forms of creativity are so immediately in the public interest as creativity in medicine. Today we are witnessing the beginnings of a rebirth of such a communion of the public and the medical scientists—a rebirth founded on partial understanding and some misunderstanding. How can we foster this renaissance; how can we narrow these errors of misunderstanding? And, if you will, why should we?

The cardinal reason, in my view, is the one already presented, namely, that creativity is never so robust as when it is an intimate part of the fabric of the times. As we have seen, this can occur even under totalitarianism, but it can be accomplished so much more easily by the community participation so natural to a free society.

How has this beginning of the communion of the public and the medical scientists come about? It has come about because both the public and the scientists have changed their attitudes.

Only a few years ago, the medical researcher lived essentially apart both from the community in which he lived and from the rest of the scientific and industrial communities. He carried on his work with the relatively simple apparatus and techniques available in such an isolated world, and there was no strong tradition of public accountability either for the questions he chose to study or for the public communication of his results. Indeed, the scientific atmosphere was such only a decade or so ago, that it almost seemed as if the more remote a particular piece of research appeared to be from any conceivable relation to the problems of the day, the greater was its scientific attractiveness.

As for the public communication of results, such scientific conduct was thought to be disgraceful. The very fact that the significance of the results might conceivably be rendered understandable for the layman was its own proof that the work itself must be pretty superficial and without any real importance.

In the years since World War II, however, the methods of the medical scientists have increased enormously in their complexity. This has had two consequences: first, it has been necessary for the scientists to work out some practicable means of communicating with each other; and, second, the development of enormously complicated apparatus and techniques has necessitated a great need for funds for medical research.

Both of these developments have had the effect of enlisting the lay public in medical scientific activities. Let us see how this increasing complexity of medical technology has had the paradoxical effect of recruiting the laity.

In contrast to the ivory tower world of a few decades ago, today the medical scientists are bringing to bear on their problems of human illness, not only their own techniques from biology, but also all the methods and all the apparatus

[1] Luncheon speech delivered at the annual meeting of the New York Tuberculosis and Health Association, April 8, 1959.

[2] For an excellent discussion of this question of the relationships of the artist and the scientist to the times and to each other, the reader should consult the recently published Rede Lecture by Sir Charles P. Snow, entitled *The Two Cultures and the Scientific Revolution*, published by the Cambridge University Press, London, 1959.

that can be borrowed from the rest of the scientific and the industrial worlds.

Advanced mathematics, physics, electronics, the composition of plastics—all subjects that were once quite foreign to the medical researcher—are now eagerly pursued by him for aid in studies of how our body works. Even the Univac-like calculators of the mathematicians are having increasingly profitable employment in research in health.

These new tools from the physical sciences are permitting medical researchers to delve ever more deeply into the individual tiny molecules that form our flesh. At the same time, other medical researchers are studying man as a whole, and indeed are studying the medical problems of localized communities of people as a whole. Thus, medical research today is spreading out toward two opposite poles at one and the same time.

Toward one pole are the studies of the most minute units of living matter; toward the other pole, the studies of how grown-up people react to their diseases.

One immediately realizes that it is not too great an exaggeration to say that the experts with such widely different stores of knowledge are learning to communicate with each other as laymen. And they all realize that it is very much to be desired from the standpoint of the fruitfulness of their research that they *be* able to interchange ideas and results with each other. In large measure, when Dr. Schmidt[3] and his associates working with their molecules wish to interchange with Dr. Deuschle[3] and his group working with a whole community, they obviously have to exchange ideas in a general language intelligible to laymen. Once the scientists found themselves talking in this general sort of language, it was very easy indeed for the public to listen in—and eavesdrop they have—because creativity in something as close to human welfare as medicine is an exciting business.

In other words, the individual scientists from the many different fields today have been forced into the position in which they have learned to choose the central core of their most complicated research and present it in terms which the rest of us can understand. Not only does this mean that the scientists from the many different fields are now beginning to be able to understand each other, and hence borrow ideas and methods back and forth; what is more, the lay public, which is all of us, is beginning to get a useful understanding of what these matters are all about. This has led to the involvement of the lay public in medical research on a widespread basis. The public now has awareness of the goals of medical research, familiarity with the significance of its triumphs and defeats and, not least of all, a very considerable involvement in its financial support. For the increased complexity in the broadening of the medical sciences has understandably led to a great need for funds for research.

The scientists notoriously have no money of their own, so what could be more natural than that when they were in need they began to approach the lay public. Although I have recited this sequentially, it is obviously a circular phenomenon.

Indeed, I am not really sure whether it is the scientific community that is successfully capturing the imagination of the lay public, or whether it is the lay public that has successfully captured the scientist and is goading his thoughts and imagination. In any case, the results are the same, namely, widespread lay public involvement in the problems of medical research to an extent that amazes visitors to our shores, and a beginning appreciation that, in considering the broad aspects of medical research, we are all laymen together.

Obviously, none of this could have happened were it not for the efforts of public-spirited men and women in the sciences, in government, and as individual laymen. Moreover, without question, one of the shotguns at this wedding of the scientists and the public has been the constant prodding by the many very fine science reporters of our lay press.

A most important result of this involvement of the laymen in the affairs of medical research has been the growing realization that medical research to a considerable degree is in part a community enterprise. This is not to deny the fact that all science is international—because of course it is—but it is now just beginning to be recognized that, as the public at large has a great stake in medical research, so does the individual community fortunate enough to have such activities going on in its midst.

To return momentarily to the opening theme of creativity, it is beginning to be realized that a community fortunate enough to have creativity in the scientific world going on should support it in the same way that it supports creativity in music, in arts, in literature, and in the theatre. For, where did the scientists first turn in their

[3] Speeches presented at the morning session of the annual meeting of the New York Tuberculosis and Health Association on April 8, 1959: "Some Observations on the Use of Drugs in the Prevention of Experimental Tuberculosis (Chemoprophylaxis)," by Leon H. Schmidt; and "Tuberculosis Among the Navajo: Research in Cross-cultural Technologic Development in Health," by Kurt Deuschle.

need for more funds for medical research? Interestingly enough, it was not to the government, but to the nongovernmental members of the community.

Indeed, it is of considerable interest that the first great national health organization that solicited public funds for its activities, the National Tuberculosis Association, with its Christmas Seals, had, and still has, a fixed policy that the major proportion of the funds collected remain within each particular community. And, as we all know, we are gathered here today for the annual meeting of what is, in effect, the New York Chapter of that national community organization.

The National Tuberculosis Association blazed the trail of raising funds from the individual members of the community, and this practice has now been successfully followed by a number of other very fine organizations that have devoted themselves to improved health and the support of medical research through community appeals.

Thus, the universities with their relatively meager resources and the private foundations were joined by these powerful allies, the voluntary health organizations, founded on a community basis in the raising of funds to keep pace with the increasing complexities of the scientific effort.

It was not until World War II that our federal government came into the picture to any great extent. Since that time, however, the national government, through the Public Health Service with its National Institutes of Health, the National Science Foundation, the Atomic Energy Commission, and various other branches, has made a steadily expanding contribution to the support of medical research. Indeed, the proportionate share of the federal government now outweighs all the rest. And it is anticipated that the federal government's contribution will continue to expand in a staged manner.

In the Bayne-Jones Report issued in 1958,[4] it was recommended that by 1970 the total expenditure for medical research in the country should approximate one billion dollars, one-half of which will be supplied by the federal government. Thus a ceiling is anticipated.

Where is the other one-half to come from? In part it will come from the private foundations although, understandably enough, the foundations, especially the larger ones, are tending more and more to devote their resources to other aspects of human welfare than the problems of medical research in the strict sense of the term. In part, the nongovernmental one-half billion dollars will come from the pharmaceutical industry; but, here too, the dollar-for-dollar comparison is not so great as it might seem. For, there is a considerable duplication and overlapping in the pharmaceutical research effort because of the fact that the individual firms are in a competitive situation with each other.

In large measure, therefore, if our medical research effort is to keep pace with our expanding population and economy, the portion not supplied by the federal government will have to be furnished by the community. This will have to take the form both of increased contributions to the various voluntary health organizations and, in some cases, by local tax money, as in the case of the newly created New York City Health Research Council.

But what would be the effects if a community well-supplied with medical research facilities and scientists failed to measure up to its obligations to provide some portion of their support? Would it mean that the medical research there would be severely curtailed and that babies would start dying who could have been saved by the fruits of the research? Neither of these two things would happen, but the community would suffer a real loss, although in more subtle ways.

The medical scientists already there would continue to carry on their programs supported by funds largely derived from the outside, specifically Washington. I would be the last to propose that deriving research funds from Washington is a horrid specter with which to frighten children. Nevertheless, whether one is a latter-day Jeffersonian or Hamiltonian, there are few among us who believe that it is healthy to have *all* of a public service—and medical research is clearly a public service—supported entirely from Washington. Or indeed, from any other single place.

For, there are fashions in medical research. Neither the scientific worker nor the committees that grant him funds are immune to the pressures of these fashions, and the mutual interaction of grantee and grantor tends to perpetuate them. This would be especially the case if the great majority of the funds came from one central source. There would be a real danger that an independent-minded investigator who wished to try an approach that had not yet come into fasbion might find himself hard pressed to get support. The obvious protection against such an eventuality would be local community support for medical research to counterbalance the mis-

[4] Final report of the Secretary's Consultant on Medical Research and Education: Advancement of Medical Research and Education. Through the Department of Health, Education, and Welfare, Washington, D. C., dated June 27, 1958.

taken judgments that are bound to occur from time to time with overcentralism.

In our own community, as we all know, we have such local sources of support. These will have to be considerably increased, however, if we are to stay in balance with the projected national medical research effort considered appropriate for our expanding population and economy. It is particularly gratifying, therefore, to see that the New York Tuberculosis and Health Association has recognized this fact and has very considerably expanded its own program for the support of medical research in this community.

* * *

As mentioned previously, the receipt of research funds from the public, whether through a voluntary health agency or through government, means that the medical scientists have likewise accepted the principle of public accountability. In the case of a national agency, such accountability usually takes the form of an annual written report. When the funds are obtained from the community, however, the reports are much more often made face to face, so to speak, and frequently are made in lay terms to a mixed group of physicians and laymen. This practice is distinctly advantageous to both the community and the scientific investigators. For, in making such personal contact reports, the medical scientist is invariably drawn into discussions and consideration of the important health problems of that community. In this way and frequently only in this way does the highly specialized scholarly scientist gain that all-important first-hand contact with the major health problems of his times that is so nourishing to creativity.

The community likewise profits from this interchange, sometimes by obtaining expert advice on a specific problem, more often simply by ensuring that the medical scientists are knowledgeable about what is going on around them. This might lead a particular scientist to start studies of some problem that is being neglected in the fashionable scientific circles. At the very least, the fact that the scientists have been made knowledgeable by the community means that they discuss the importance of the emerging problem with their colleagues. And, in science, as in anything else, what is on everyone's tongue thereby becomes fashionable.

In essence, what I have been saying is that, although medical research knows no local or national boundaries, it needs and deserves local community support. In these respects it is no different from many other forms of intellectual or artistic creation. When community participation and support of medical research fail to develop, the research continues to go on. But, instead of scientists who are oriented by the community to the health problems of the day, we have army post–like islands of researchers who can become remarkably isolated, to their detriment and that of the community. What I have *not* been saying is that the total sums to be provided by the community should ever approach those that can be provided by the federal government, but merely that the community does have a responsibility to do what it can. To put it crudely, the medical scientists of your community, just like the ball club, need fans.

* * *

So much for the recent growth of understanding between the community and its medical scientists, where lie the important misunderstandings. It seems to me that there are three principal areas of misunderstanding.

The first has to do with the misconception that, if enough scientists and enough money are devoted to the study of an important practical question, it will be solved. The plain and simple truth is that the practical results of medical research—the great developments that can be applied in the treatment of human disease—in actuality "pop up" as the happy by-products of a much larger total research effort.

In order to get these by-products, which in practical terms are the "payoff," it is necessary to keep a large program going on the pure study of the way "things work" in the particular disease or diseases one wishes to attack. In other words, the successful research system is fundamentally based on there being a large number of imaginative scientists continously engaged in trying to find out what makes clocks tick—without their having to be concerned at all with the manufacturing problems of the clock business. And when the public is contributing to the support of research, it should realize that it is supporting scientists who quite properly operate in this way, i.e., the pursuit of knowledge for its own sake.

The second great area of misunderstanding has to do with the misconception that, if medical research is pursued with sufficient vigor, and as each separate major disease comes under effective control, there will eventually come a day when we will all be essentially free of disease. Unfortunately, it doesn't work that way. Disease is virtually the natural state of a community of men or, for that matter, of a group of lower animals or plants. When we learn to drive one major disease from a community we generally do so by changing the environment of that community, and the very environmental change itself

favors the development of other menaces to our health.

For example, fifty years ago, in our cities we were subject to certain diseases that were helped to spread by the flies that multiplied freely in the stables on virtually every block. Attempts by the health authorities to control the flies were futile; but when Henry Ford introduced low-cost automotive transport, the stables were abandoned and the flies were no longer such a menace.

But the automobile gives us 40,000 deaths on our highways each year aside from its probable contribution to disease of our lungs by means of the unexploded high octane gasoline and the pulverized rubber released in such quantities into the air we breathe. No, in man's battle against disease via medical research, it is naive to think in terms of final victories. The best we can do is to get major armistices here and there until the conflict breaks out someplace else.

For health is not freedom from disease. Instead health is to be defined as the degree to which a person is able to operate effectively within the circumstances of his particular heredity and environment. As many of you know, our distinguished scientist Dr. René Dubos has developed this theme at length in his extraordinarily perceptive book, *Mirage of Health*.

The third cause of misunderstanding of medical research by the community is the notion that, if X disease is brought under control, we will save so many dollars each year. The fallacy of this argument should be evident from what has just been said. All too often, however, the community obtains this false notion from well-meaning groups seeking to raise funds for medical research. In actuality, in man's fight by medical research against disease, as in his fight against ignorance by education, it is necessary to keep spending money just to stay even—to say nothing of actually forging ahead.

* * *

To say that there are no final victories is really not to paint too gloomy a picture. For, to a very real extent, what has been done by medical research and the application of its results by the community has been to substitute diseases of middle and old age for the diseases that formerly killed or crippled our young. In this way the community has seen to it that our children get their chance to make their run through life's most productive years.

Much more remains to be done. Among other things there should be an expanded research effort on the usually chronic incapacitating illnesses of middle life, for it is in this area today that our community is carrying its heaviest burden from the standpoint of health. And, with full community participation along with increased financial support from outside, it should be possible eventually to cause appreciable modifications in the disease patterns of these middle years.

To sum up, it can be said that all living things, including man, are inevitably disease ridden and mortal; but within these immutable limits, some diseases are preferable to others. In the selection of which diseases we have, there is some element of choice, for the occurrence of particular diseases depends to a considerable degree on the forces we set up or tolerate in our particular community.

WALSH MCDERMOTT
New York, New York

REPORT ON THE SECOND ASPEN CONFERENCE ON RESEARCH IN EMPHYSEMA[1,2]

THE MORPHOLOGIC BASIS OF PULMONARY MECHANICS

Aspen, Colorado, June 12–14, 1950

THE CONFERENCE STEERING COMMITTEE[3]

Encouraged by the 1958 Aspen Conference (1), but conscious of its chief defect—lack of focus on a specific aspect of the emphysema problem—the 1959 Conference was planned with emphasis on a single topic related to pulmonary emphysema, namely, the relationship between the structure and mechanical properties of alveolar tissue. The participants were chosen and the agenda planned in order to stimulate a maximum of effective communication between specialists in different fields. The Steering Committee was particularly anxious to have the physiologists see the geometry of the alveoli whose mechanical properties they had been deducing, to allow the morphologists to appreciate the import of physiologic deductions, and to stimulate the biochemists to provide more information on the composition of the lung.

This report will summarize both the formal presentations of the first day and the highlights of the informal discussion periods during the following two days.

I. THE MORPHOLOGY OF ALVEOLI

THE SIZE OF PULMONIC ALVEOLI OF COMMON LABORATORY ANIMALS AND MAN[4]

W. STANLEY HARTROFT[5]

Shortly before his death, Sir Frederick Banting arranged that Professor C. C. Macklin should undertake supervision of the writer in an investigation suggested by the late Sir Joseph Barcroft, Cambridge, England, to determine the relative size of pulmonic alveoli of common laboratory mammals and man. Although portions of this work have been presented in abstracts, and in a privately circulated extramural report (Sub-Committee on Physiological Aspects of Chemical Warfare, Canada), results have not been published in readily accessible form.

Results are based on the total of 132,000 measurements of 47,800 alveolar outlines selected from 25-micra microsections of lungs of man, cat, rabbit, guinea pig, monkey, dog, baboon, goat, rat, and mouse.

Animals' lungs in the intact (unopened) thorax were fixed by prolonged intravascular perfusion with Bouin's picroformol. To effect thorough stiffening of the lungs in the state of expansion, a perfusion period of three weeks or even longer proved to give the best results. Great care was exercised in removing lungs not to deform them in any manner. They were immersed in 3 per cent formalin and never allowed to lie unsupported by liquid lest their weight induce distortion and collapse of dependent parts. Blocks of uniform size for paraffin sections were chosen from standard regions of each of seven lobes and, after paraffin embedding, were sectioned in a plane perpendicular to the pleurae. Measurements indicated that by this technique shrinkage of tissue was standardized to a reasonably uniform extent (15 per cent). A section-thickness of 25 micra was found optimal for mensurational purposes, and Ehrlich's hematoxylin and eosin the most suitable stain. More than 5,000 sections were prepared and stained by this or various other methods. Three selected dimensions of alveolar outlines in these sections were defined and adopted for measurement using camera lucida tracings obtained by a uniform procedure. Areas of outlines were meas-

[1] Prepared and edited by Drs. Giles F. Filley, George C. Bower, and Roger S. Mitchell.

[2] From The Colorado Foundation for Research in Tuberculosis and the Department of Medicine, University of Colorado School of Medicine, Denver, Colorado.

[3] Drs. John R. Durrance, C. Wesley Eisele, Ben Eiseman, Giles F. Filley, Fred Harper, Gardner Middlebrook, Roger S. Mitchell, Solbert Permutt, and Mr. Ronald Miller.

[4] From the Department of Pathology, Washington University School of Medicine, St. Louis, Missouri.

[5] The writer wishes to express his appreciation to Professor C. C. Macklin for his direction and many helpful suggestions in collecting these data; and to the Department of National Defense, Army, and the National Research Council of Canada for financial support. The experiments were carried out in Professor Macklin's laboratories in the Department of Histology, University of Western Ontario, London, Ontario, Canada.

ured in some instances with a planimeter. Two hundred outlines per lobe (or a total of 1,400 per lung) were so prepared and documented on long strips of adding-machine paper.

In the case of man, these methods were of necessity slightly modified. The lungs were fixed *in situ* by intratracheal rather than by intravascular perfusion, and the seven blocks were chosen from lobar regions of the five lobes to correspond to the seven lobes of animals' lungs, using Huber's bronchopulmonary segmental classification as a guide.

The resulting data indicated that the mouse possessed the smallest alveoli of the species studied and man the largest, depth-width averages of their alveolar outlines being 38.69 and 166.11 micra, respectively. Alveolar sizes of the remainder ranged in the following ascending order: baboon, dog, goat, guinea pig, monkey, rabbit, and cat. Although a rough correlation with body size was noted, several exceptions were found. For example, alveoli of the cat are larger than those of either dog or baboon. The available data permit calculations not only of areas of outlines of sectioned alveoli, but also, in some cases, of volumes of "mathematically reconstructed" alveoli (Cole-Hartroft method). For instance, calculations of the latter type indicated that the average volume of pulmonic alveoli of man averaged ten times that of dog.

MORPHOLOGY OF THE ALVEOLAR WALL IN HUMAN LUNG[6]

RICHARD F. BAKER AND CLAYTON G. LOOSLI

The structure of the alveolar wall in the lung of man has been studied using ultra-thin sections and electron microscopy. Tissue was obtained surgically from children and adults. Fixation was done by immersion in 1 per cent buffered osmium tetroxide. The time between excision and immersion in the fixative was never longer than one minute. In thin sections a continuous endothelial capillary lining was observed with numerous interlocking cellular junctions. The basement membrane, which appeared homogeneous, was interposed between endothelium and a continuous cytoplasmic layer that lined the capillary on the air side. Cellular junctions were never seen in the external cytoplasm, suggesting that the layer may form a syncytium over the alveolar wall. Gas or fluid transport across the boundary is possibly represented by numerous vesicles which were seen in the cytoplasm on both sides of the basement membrane. Macrophages with osmophilic inclusions were seen both in the air space and resting on the alveolar wall. Cells possessing the morphologic characteristics of septal cells were observed as part of the external cytoplasmic layer. They were distinguished by a protrusion into the air space and the possession of large spherical nuclei. In all cases these cells were definitely outside the basement membrane. The distribution of collagen in the wall was studied, using phosphotungstic acid staining of the sections to enhance contrast. Elastic tissue was intimately associated with collagen and took the form of homogeneous fibers and sheets.

[6] From the departments of Medical Microbiology and Medicine, University of Southern California School of Medicine, Los Angeles, California.

IN VIVO ARCHITECTURE OF BLOOD VESSELS SUPPLYING AND DRAINING ALVEOLI[7, 8]

WILLIAM H. KNISELY[9, 10]

Several investigators, including Wearn and associates (1926, 1927, 1928, and 1934) and Irwin and associates (1953 and 1954) have studied living mammalian lungs. In 1952, Dr. Irwin kindly demonstrated his technique to me.

These observations are generalizations based upon examinations of the living lungs of some 50 dogs, 300 cats, 600 rabbits, a few guinea pigs, hamsters, rats and mice, and 2 rhesus monkeys. All were anesthetized, usually with sodium pentobarbital. Most were open chest preparations. Quartz-rod transillumination and a Leitz dissecting microscope at 16 to 150× or a binocular monobjective microscope with water immersion lenses at 500 and 900× were used. Observations were limited to the surfaces and edges of lobes; necessarily, the shapes of these alveoli and their vascular supply are not completely representative of the shapes or blood supply in the interior.

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[8] This work was supported by grants from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association; from the Office of Naval Research (NR114-065), Department of the Navy, to the Medical College of South Carolina; by a grant from the American Heart Association to Duke University; and by funds from the Duke University Center for the Study of Aging (in turn supported by the Heart and Mental Health Institutes, U. S. Public Health Service).

[9] This study was partially carried out during tenures of an American Heart Research Fellowship and a Senior Research Fellowship (SF-65), U. S. Public Health Service.

[10] Present address: Department of Anatomy, University of Kentucky Medical Center, Lexington, Kentucky.

Alveoli vary in size and shape from species to species. *In vivo* it is difficult to state with certainty what is one "alveolus" because of the folds in the walls of the "sacs"; and it is not possible to state, based upon histologic terminology, whether a whole sac or only the portions relatively "enclosed" within the folds in the walls are "alveoli." In spite of these limitations to a precise *in vivo* morphologic definition, the term *alveolus* is used.

The intercapillary distance on surface alveoli is approximately six times the width of an individual capillary. The diameter varies along pulmonic capillaries; they are a little wider than systemic capillaries, and 12 to 14 microns in diameter is usual.

The architecture of vessels supplying alveoli differs from the simple dichotomous branching of systemic arterioles supplying capillary beds. While the largest pulmonary arteriole or venule visible to the naked eye is approximately 150 micra, a few up to 300 micra have been studied. These arteries taper rapidly; their branches may be capillary-sized or much larger. These capillaries branch radially at nearly 90° and supply the alveoli contiguous to the artery, while arterioles up to 100 micra also branch off and in a similar manner supply more distant alveoli.

One alveolus may be supplied by more than one arteriole; one arteriole supplies many alveoli, and capillaries may cross more than one alveolus, thus forming networks across several alveoli. Consequently, capillary anastomoses occur among arterioles.

Alveolar vascular drainage was studied, although most venules and veins turn deep into the lung. Capillaries unite forming venules; however, capillaries also enter veins at approximately 90° from contiguous alveoli, proximal to the entrance of vessels as large as 150 micra.

The vascular architecture near alveoli bears a relation to physiology and pathology. In arteries, capillaries, and veins near alveoli, a positive pulse, a nearly linear flow, or a negative pulse may be seen. Flow across alveoli can be reduced or stopped by increasing intratracheal pressure. When arterioles are embolized, blood can continue to flow (*a*) up against emboli and laterally out the radially branching capillaries; (*b*) around capillary anastomoses and out the terminal end of the artery; or (*c*) retrograde in the portion of the artery distal to the embolus.

Discussion of Papers on Morphology

In the discussion which followed these introductory papers on anatomy, the laborious two-dimensional measurements of Hartroft provided a firm although limited basis for the development of *quantitative pulmonary morphology*. Drs. Hollis Boren (Houston, Texas) and Philip Pratt (Columbus, Ohio) were able to demonstrate certain clear-cut advantages of studying air-dried lungs in three dimensions, not only for alveolar *size*, but also for alveolar *arrangement* in *space*. The question of whether alveolar walls are flat or rounded in life was left open because of the gratifying agreement among all morphologists that, to apply Dr. Isidore Gersh's comment, "No histological artefact has been completely explained, except for such simple ones like solution of fat droplets in lipid solvents."

This matter of artefacts clearly applied to electron microscopic visualizations of alveoli. The problem of whether or not the normal alveolar wall is completely epithelized involves the question of how much of the alveolar lining demonstrated by electron microscopy is artefactual. If this much doubt exists about cellular structures, how much help can the physiologists expect from the morphologists in determining the anatomic character of a mucoprotein-like film (which Dr. Hartroft referred to as the "Terry fluid") lining the alveolar walls? It seemed clear that highly sophisticated morphologic techniques would be required to demonstrate noncellular structures in the lung.

Dr. Knisely's *in vivo* demonstration of how at least some of the capillaries appear in nonbreathing alveoli raised but did not answer the important questions of how capillaries are normally distributed in mammalian alveolar walls. Several physiologists emphasized that the technique used, although elegant in many respects, still involved grossly unphysiologic disturbances in gaseous environment, electrolyte balance, and the hemodynamic factors affecting capillary flow.

Dr. Vernon Krahl (Baltimore, Maryland) confirmed Dr. Knisely's demonstration that the arterioles supplying capillary beds in the lung "give off at about 90° short branches which supply the alveoli contiguous to the artery" and that this arrangement seems to be typical in mammals.

II. THE HISTOCHEMISTRY OF THE RESPIRATORY UNIT

SOME NON-CELLULAR STRUCTURES OF THE LUNG[11]

ISIDORE GERSH

The lung is considered for the purposes of this talk as an organ consisting primarily of connective tissue. An important problem then is the con-

[11] From the Department of Anatomy, University of Chicago, Chicago, Illinois.

tribution of each of the connective tissue components to the elastic properties of the lung. Cells could be eliminated. There remain, then, ground substance of connective tissue, including the homogeneous component of basement membrane, reticular and collagen fibers, and elastic fibers.

The ground substance of connective tissues, including basement membranes, consists of a two-phase system. This system comprises water-rich, colloid-poor vacuoles separated by a dense phase which is water-poor. The former may be visualized by the intravenous administration of sodium ferrocyanide; the droplets which contain the ferrocyanide may be observed with the electron microscope or, when they are sufficiently numerous and occur in clusters, with the light microscope. Variations in the two phases take place during aging, following treatment with certain hormones, and in inflammation. Whether or not the ground substance of the lung responds to these or other factors has not been determined.

The homogeneous component of the basement membrane is part of the dense phase, and encloses collagenous fibrils. The homogeneous and fibrillar components may be seen clearly in electron micrographs of the basement membrane of fat cells (Chase), but the intimate relations in pulmonary basement membranes are not so clear.

Additional studies with the electron microscope show that, in lungs prepared by freezing and drying, the surface of pulmonary alveoli is normally covered by a layer of noncellular homogeneous material, except in frogs. In mammals, the cells of the alveolar wall do not seem to be contiguous, as they are in the frog, and are separated from the air space by the surface layer referred to above (Chase). Whether and to what extent the surface film corresponds with the "substance X" of Clements is not known.

Elastic fibers of frozen-dried mouse lung were stained differentially with orcein (Chase). This method of staining results in sufficient contrast so that their fine structure may be studied. Elastic fibers are resolvable into smaller structures with the finest fibril about 80 Å in diameter. These seem to have a periodicity of about 150 Å. Their distribution in visceral pleura, alveolar wall, and bronchi was studied.

It is possible that ground substance, collagen fibrils (as occurring particularly in reticular fibers) and elastic fibers all contribute to the elastic properties of the lung; the homogeneous surface layer of the alveolar walls may also be involved.

CHEMICAL COMPOSITION OF PULMONARY ELASTIN OF THE DOG[12]

VERNE D. HOSPELHORN AND MARTIN J. FITZPATRICK

In view of the speculation about alterations of elastic fibers in emphysematous lungs, detailed studies of the composition and structure of elastic tissue would appear to be of some interest. This report represents some preliminary results found on chemical analysis of elastin from dog lungs.

The methods which have been used in isolating elastic tissue (elastin) are: autoclaving of aqueous or dilute acetic acid suspensions of connective tissue or heating the connective tissue in 0.1 N NaOH solutions. The residue remaining after these treatments is called elastin. It has been recognized that the rather drastic conditions in these procedures probably produce chemical changes in the native elastic fibers. For this reason, we have developed an alternate method of isolating elastin from tissue in which the proteolytic enzymes trypsin and collagenase are used to digest away the extraneous protein material.

This second procedure for isolation of elastin is as follows: Lungs from healthy dogs are freed from major bronchi and blood vessels and blended with water in a Waring Blendor. The connective tissue with some cellular debris is collected by centrifugation and then washed several times with water. This material is then extracted with sodium chloride solution to remove adhering nucleic acids and is again washed several times with water. The residue is dried and extracted with *n*-butanol to remove lipids. The product from these operations is called crude connective tissue (C.C.T.).

Crude connective tissue is subjected to alternate digestions with trypsin and collagenase until no protein material is liberated. The insoluble product is called "enzyme" or "E-elastin." In order to have a basis for comparison with elastins from other tissues and with E-elastin, crude connective tissue is also heated with 0.1 N NaOH to yield what we call "base" or "B-elastin."

Some results of analyses of these preparations are shown in the following text table. Values are expressed in per cent of total elastin.

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[12] From the Department of Medicine, Lerrigo Memorial Laboratory, University of Kansas City Medical Center, Kansas City, Kansas.

	E-elastin	B-elastin
Nitrogen	12.6	14.1
Phosphorus	0.2-0.3	<0.05
Ash	Ca 0.4	Ca 0.4
Amino Acids		
Isoleucine	3.8	2.7
Leucine	6.4	6.2
Hydroxyproline	1.4	1.7
Proline	13.05	13.7
Phenylalanine / Tyrosine	8.9	8.4
Valine	9.3	12.0

The low hydroxyproline content of these preparations is particularly significant since this indicates no contamination by collagen. E-elastin contains considerably more aspartic acid, threonine, and serine than does B-elastin. In addition, an unidentified yellow component is found in the hydrolytic products of B-elastin which is not present in the hydrolytic products of E-elastin. Both hydrolyzed preparations contain unidentified hexane-soluble material.

Neither elastin preparation is attacked by trypsin or collagenase but B-elastin is much more susceptible to digestion by papain and elastase than is E-elastin.

Explication of the reasons for the differences between these preparations should yield useful information on the precise composition and structure of elastic tissue. Additional information regarding the composition and structure of elastin from normal and from emphysematous lungs should tell us whether biochemical changes in elastic tissue are associated with emphysema. Such changes, if any, may well prove to be more subtle than simple quantitative alterations.

THE STRUCTURE OF THE RESPIRATORY UNIT IN EMPHYSEMA, COMMON AND UNCOMMON TYPES[13]

EDWIN R. FISHER

Knowledge of the structure of the respiratory unit of the lung (the respiratory bronchiole and its lateral and peripheral ramifications) appears essential to an understanding of the pathogenesis of both the common (vesicular) and uncommon (bronchiolar) types of emphysema. Utilizing serial sections of both normal and emphysematous lungs, measurements of the component parts of the respiratory units have provided significant information in this regard.

In emphysema of the vesicular type, which affects principally the distal portion of the respiratory unit, the elastic fibers of alveoli are quantitatively and qualitatively similar to those of normal lungs. It seems unlikely that a genetic defect in elastic tissue is etiologically responsible for this type of emphysema. On the other hand, in bronchiolar emphysema involvement of the proximal portion of the respiratory bronchiole is paramount. The alveolar elastica is markedly hypertrophied, although qualitatively, as disclosed by histochemical techniques, similar to that of normal lung. This alteration in alveolar elastica plus increase in septal collagen and fewer but larger alveoli ("respiratory hypoplasia") are believed to determine whether emphysema will be of the vesicular or bronchiolar type. The wall of the proximal respiratory bronchiole represents an inherent focus of weakness in the respiratory unit since support is provided only by thin, oblique, and discontinuous bundles of smooth muscle and delicate longitudinal elastic fibers. Furthermore, the proximal respiratory bronchiole has a muscularis which is partly absent on one side, that which is devoid of rudimentary alveoli. On the other hand, the distal bronchiole is supported by a strong net of elastic fibers.

Discussion of Papers on Pulmonary Histochemistry

This additional material began to open up many new avenues of thought. In particular, Dr. Gersh's demonstration that the intercellular tissue is composed of an organized ground substance led to the concept that the lungs consist essentially of tissues which hold the airways and blood vessels in place. The very existence of a ground substance of the lung was new to many conferees. The possibility that disturbances in the ground substance might have some bearing on the nature of emphysema provides a totally new area for investigation of the disease.

It has long been believed that elastic tissue disturbances play an important role in emphysema. Are they primary or secondary? Can chemical differences be found when morphologic differences are not evident? Are changes in elasticity due to alteration in the elastic tissue? Or are they due to changes in one of the other connective tissues? A unique approach to the problem of determining how elastic structures are distributed in three dimensions in the lungs was demonstrated by Dr. Robert W. Carton (Chicago, Illinois), who has succeeded in completely isolating the elastic tissue of human lungs by formic acid digestion of all other tissue. In emphysematous lungs the elastic fibers were sparse and abnormal in arrangement and continuity.

[13] From the Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania.

III. THE MECHANICAL PROPERTIES OF ALVEOLI

ELEMENTARY CONSIDERATIONS CONCERNING INFLUENCE OF SURFACE TENSION ON THE MECHANICAL BEHAVIOR OF THE LUNGS AND, IN PARTICULAR, THE STABILITY OF THE AIR SPACES[14]

JERE MEAD

What is meant by the word tension (figures 1, 2)? Pressure may be thought of as force operating at right angles to a surface, and has the units of force per area, e.g., grams per square centimeter. Tension may be thought of as force operating in the same plane as the surface. For example, tension is applied to a window curtain by way of the cord. The amount of tension is expressed as force per length. In the example, if the downward pull on the curtain equalled 1,000 gm. and the curtain was 100 cm. wide, the related tension in the curtain would be 1,000 gm. ÷ 100 cm. = 10 gm. per cm. A convenient unit for surface tension is *dynes* per cm. (1 gram equals 980 dynes).

What is surface tension (figure 3)? The surface of any liquid is under tension. This results from intramolecular forces of attraction between the molecules of the liquid. At a distance from the surface these forces are equal in all directions. But molecules at the surface have a preponderance of near neighbors in the direction of the liquid. Thus, there is an unbalanced inward attraction on them which results in a tendency for the surface to decrease in area. This tendency can be measured in terms of the tension developed in the surface.

The relationship between the tension in the wall of a sphere and the pressure within (figures 4–7): The gas-liquid surfaces lining the air spaces within the lungs are curved. A *curved surface* under tension implies a pressure difference between the two sides of the surface. We will develop this relationship for a sphere. Increased tension in the wall of a sphere results in increased pressure inside relative to that outside the sphere. The relationship between tension and pressure was shown by Laplace to depend on the radius of the sphere as follows: $P = 2T/R$. One derivation of this expression is shown in figures 6 and 7. At equilibrium the tendency of tension to contract the sphere is exactly counterbalanced by the tendency of pressure to expand it. This can be expressed in terms of the balance of forces operating along any circumference of the sphere. The total force operating at right angles to such a circumference is equal to the product of the tension and the circumference. This force may be thought of as

[14] From the Department of Physiology, Harvard School of Public Health, Boston, Massachusetts.

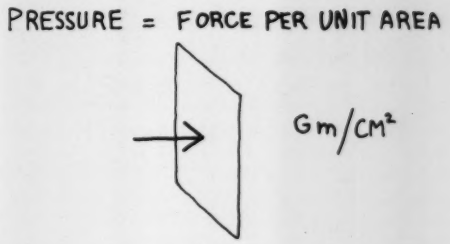

FIG. 1

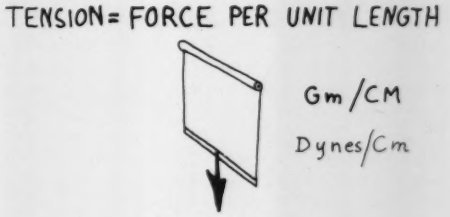

FIG. 2

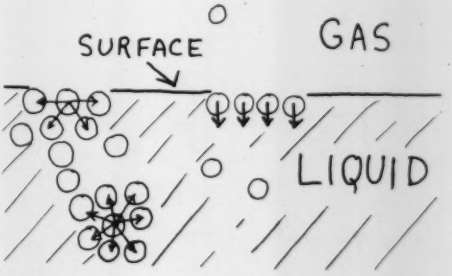

FIG. 3

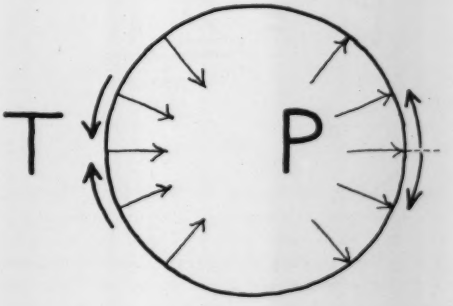

FIG. 4

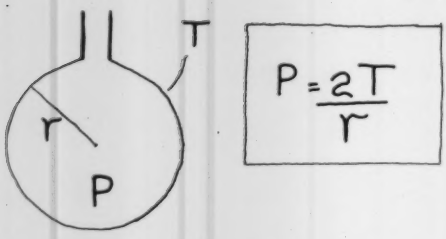

FIG. 5

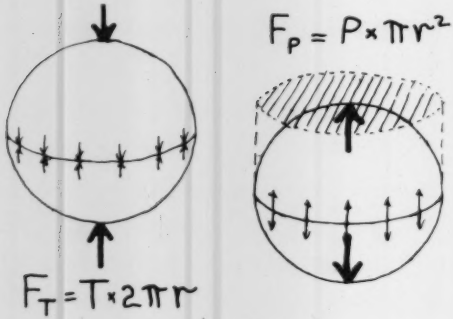

FIG. 6

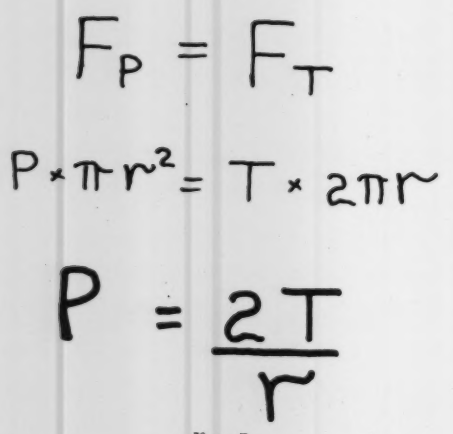

FIG. 7

operating at the "poles" of the sphere, "keeping the hemispheres together." There is an equal and opposite force developed by the pressure within the sphere tending to separate the hemispheres. This force is the product of the pressure and the cross-sectional area. When these opposing forces are equated, the Laplace relationship is obtained.

The pressure-volume relationships of a bubble on a tube (figures 8, 9): The expression P = 2T/R can be used to help predict the pressure-volume relationships of a soap bubble blown on a tube. For present purposes we will consider the film as having only one surface, the surface tension of which is *constant* no matter how large the bubble. Starting with a single film across the end of the tube, with a very gentle initial application of pressure the surface would be slightly curved and would approximate the shape of a small part of a large sphere such as is indicated by the dotted line. As a little more gas is forced into the tube, the radius of the bubble would *decrease* to a minimal value equal to the radius of the tube; with further inflation the radius would increase again. A graph of pressure against bubble volume would have the shape indicated in figure 9. According to the expression P = 2T/R, pressure would increase to a maximum and then fall progressively as volume is further increased.

The behavior of bubbles "in parallel" (figures 10, 11): It is particularly instructive to consider the behavior of two bubbles on a "Y" tube. Suppose that the two bubbles are inflated as indicated and their common airway closed off (indicated by the line across the tube opening). What would take place? Intuitively one might expect the larger bubble (P_2) to decompress into the smaller one (P_1), until the two were equal in volume. It may readily be seen from Laplace's expression and the assumption of constant surface tension that this would not be the case. The bubble with the smaller radius would have the higher internal pressure. As a result there would be a pressure difference producing flow from the smaller into the larger bubble. Flow would continue until the smaller bubble had emptied, or until each bubble had the same radius of curvature, as shown in figure 11.

This result may be extended to the behavior of more than two bubbles. The point to be made is that any such system of bubbles connected in parallel is inherently unstable. The lung consists

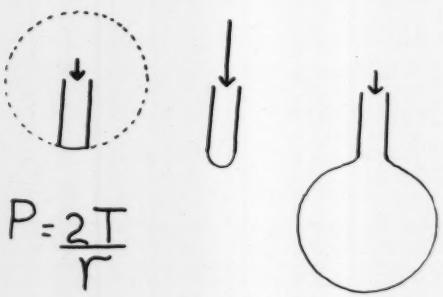

FIG. 8

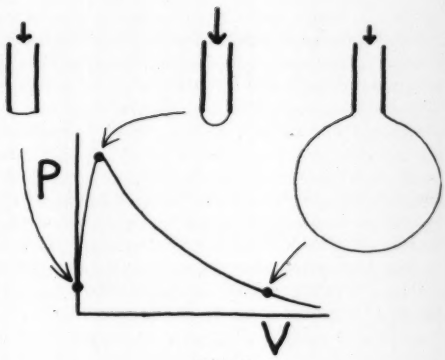

FIG. 9

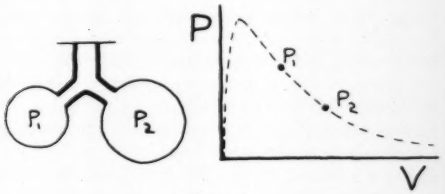

FIG. 10

of countless air spaces in parallel, all sharing a common airway. From the standpoint of its internal surface it is like a foam, but a truly remarkable foam with all of its "cells" interconnected. In itself such a foam would be highly unstable. The smaller cells would empty into the larger cells, and this process would continue until one large air space remained. Clearly the lungs' internal surface in itself should be unstable. What confers stability to the air spaces? Three stabilizing influences are recognized. These are illustrated in the remaining figures.

1. The stabilizing influence of the tissue elements surrounding the air spaces (figures 12, 13): Considered independently, the contribution of the surrounding tissue to the pressure-volume behavior of the air spaces must have the general shape indicated at the upper right in figure 12. That is, as volume is increased, pressure always increases and *never decreases.* (This statement is based on the known behavior of fluid-filled lungs in which surface forces are minimized.) The overall pressure-volume behavior of such a unit, including both the surface and tissue contribution, could be developed by summing up the surface and tissue pressures at each volume and would have the general shape shown in the lower right of figure 12. This is redrawn on a larger scale in figure 13. To the left, that is, at low volumes, the surface properties predominate in determining the pressure, while at high volumes the tissue pressure predominates.

The critical point in this graph is indicated with an arrow pointing at the valley of the curve. At higher volumes the unit would be stable; at lower volumes it would be unstable and would collapse. To arrive at this conclusion it is helpful to consider the behavior of the unit as it is inflated from the collapsed state by controlling pressure and observing the resulting volume changes. There would be an initial phase in which volume and pressure both increased. (This corresponds to the region where the bubble radius is decreasing as in figure 9.) At any point in this phase, if pressure were held constant, volume would remain constant. Any increase in pressure would be met by a sufficient increase in volume to permit an equal opposing pressure. As pressure is further increased, a point would be reached at which the unit would have to increase in volume a great deal before equilibrium was established. (This is along the upper dashed line in figure 13.) Once this sudden step had taken place the system would again be stable. All small pressure changes would be associated with correspondingly small volume changes. If now pressure were decreased in small steps, volume would decrease in corresponding steps until at a certain point the next step down in pressure would be associated with a sudden

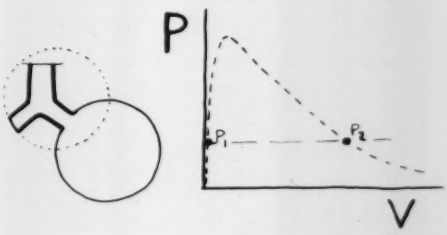

FIG. 11

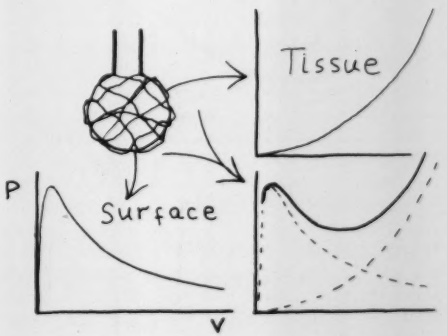

FIG. 12

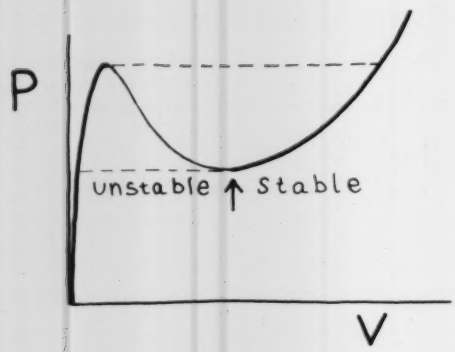

FIG. 13

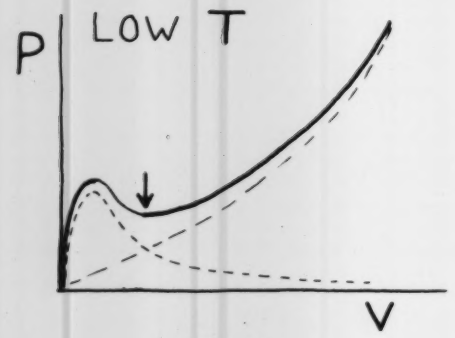

FIG. 14

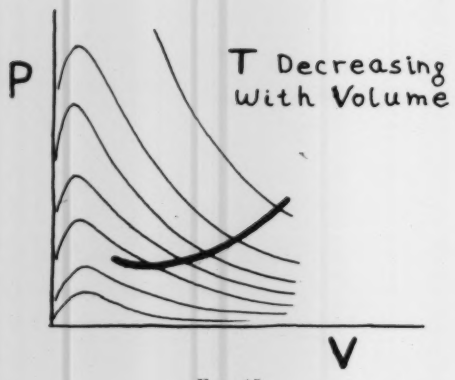

FIG. 15

volume reduction along the lower dashed line to the left. As long as pressure is the controlled variable, there is a particular volume range within which it is impossible to maintain the volume constant. This is the region where pressure and volume change in the opposite sign, i.e., the slope of the line, is negative. In this region surface tension predominates and the unit exhibits the instability of the soap bubble. At higher volumes, however, tissue forces predominate and the unit is stable.

In a system of units such as these in parallel, at high pressure all of the units would be in the upper stable range. There would be a considerable range of pressure below this high level over which the units would remain stable. As pressure was further decreased, however, the critical volume would be reached for some units and they would collapse. Finally, all of the units would be collapsed.

2. The stabilizing influence of a low surface tension (figure 14): It is apparent that a lowered surface tension would tend to displace the critical volume downward, thus extending the stable range. This is illustrated in figure 14. Recent work has shown that the surface tension of the internal surface may indeed be remarkably low (2).

3. The stabilizing influence of a surface tension which decreases as lung volume decreases (figure 15): Clements has evidence that the lung internal surface has a tension that falls off markedly as surface area decreases (3). He points out that this property is sufficient in itself to explain the stability of the air spaces. This is shown schematically in figure 15. The light lines indicate a family of pressure-volume curves for bubbles at various constant surface tensions—the higher the curve the higher the tension. The heavy line describes the pressure-volume relationship of a bubble, the surface tension of which falls sufficiently rapidly to permit a positive pressure-volume slope which is the required condition for stability.

EFFECTS OF INTRINSIC SURFACE ACTIVE MATERIAL ON THE MECHANICAL PROPERTIES OF THE LUNGS, WITH SPECIAL REFERENCE TO STABILITY OF THE ALVEOLAR STRUCTURE[15]

JOHN A. CLEMENTS

Histochemical studies, air and saline inflations of lungs, effects of nonionic detergents on lung "elasticity," and physicochemical studies of extracts of lungs suggest that another member should be added to the alveolar-capillary membranes, a surface film of mucoprotein. This film, probably monomolecular, appears to form spontaneously by adsorption to the air surface and denaturation of proteinaceous material, as do films on other protein solutions. It is unusual thus far (together with nasal mucus and sputum) in

[15] From the Directorate of Medical Research, U. S. Army Chemical Warfare Laboratories, Army Chemical Center, Maryland.

lowering surface tension to values below 10 dynes per centimeter on reduction of the area of the fluid surface, and apparently consists of a substance or complex of substances not previously studied by the techniques of surface chemistry and physics. It has been given the interim name, substance X.

An effect of substance X at the fluid surface is to increase the coefficient of elasticity of the surface and to stabilize the alveolar structure. Since film formation proceeds at finite speed, it is possible experimentally to vary the minimal volume from about 5 to 50 per cent of maximal lung volume. The phenomenon of air-trapping in excised lungs depends on static surface forces in addition to the more commonly mentioned dynamic forces. A simple cylinder-hemisphere model for the terminal air passages helps to clarify possible mechanisms of trapping when film elasticity is taken into account.

On the basis of this model and of the known properties of films produced from extracts of lung tissue, it is suggested that modification of the films at the air surface of the lung parenchyma, so as to lower surface tension in the alveolar structure or to raise it in the distal members of the conducting airways, may result in air-trapping. Yielding of the alveolar structure under prolonged dilatation, accompanied by asphyxial and ischemic deterioration of the tissue, may result finally in the morbid anatomy characteristic of chronic, diffuse, obstructive pulmonary emphysema.

MECHANICAL FACTORS DETERMINING ALVEOLAR CONFIGURATION[16]

EDWARD P. RADFORD, JR.

The configuration of the terminal units of the lungs depends primarily on a balance of forces arising from deformation of connective tissue and the presence of the air-liquid interface. The mechanical importance of the latter is indicated by the markedly altered pressure-volume characteristics of excised gas-free lungs when surface forces are eliminated by filling with saline instead of air. To determine the relation between mechanical properties of the lungs and the anatomic arrangement of the terminal units, studies were carried out in excised rat lungs whose pressure-volume characteristics were measured at the same time as photographs of the lung surface units were made. When gas-free lungs were inflated with air, the diameters of the first units visible were large (alveolar ducts?) and, as filling continued, progressively smaller units were superimposed until at a maximal inflation the alveoli had appeared as small (50 μ diameter) projections from the underlying spaces. With deflation, however, the alveoli remained as discrete units whose diameters only slightly decreased as the lung volume decreased markedly. With complete deflation many alveoli retained trapped bubbles of gas. This difference in geometry of filling and emptying was associated with marked static hysteresis of the pressure-volume curves. These observations have been confirmed in living animals.

Both the difference in geometry of emptying vs. filling and the pressure-volume hysteresis could be nearly completely eliminated by filling and emptying the lungs first with 0.5 per cent Tween® 20 in saline. Under these conditions Tween 20 evidently displaced the molecular layer normally lining the alveolar surface, leading to a higher surface tension than normal during deflation. These results indicate that special properties of the lung surface layer, particularly the effect of changes in lung surface area on the surface tension coefficient, corresponding to similar effects observed by Clements and associates with lung extracts, probably play a major role governing the geometry of the terminal lung units at the normal breathing level. This property of the surface apparently not only contributes to the stability of a potentially unstable mechanical system, but also aids in preventing the formation of fluid films across the mouths of the alveoli. Stability of the configuration of the lungs may also be conferred by the trabeculated nature of the alveoli on the alveolar sacs; this geometry could permit an increase in lung volume to occur with no change, or possibly even a decrease, in surface area of the units.

Also based upon these observations, the following tentative conclusions seem justified at the present time: (*a*) Most of the volume change of the lungs which occurs during normal breathing takes place in the alveolar sacs rather than in the alveoli themselves. For this reason in the tidal volume range the major elastic deformation takes place in circumferential bundles of elastic fibers found at the openings of the alveoli into the alveolar sacs (seen as knoblike projections into the alveolar sacs on histologic sections). Functional integrity of the primary lobule may depend to a considerable extent on the elastic properties of these fibrous bundles. (*b*) From studies of gas exchange it appears highly unlikely that a large fraction of the lung is collapsed or nonfunctional at resting lung volume. Trapping, when it occurs, probably takes place by the formation of fluid films over the mouths of alveoli or in the alveolar ducts. It is not likely that the formation of these

[16] From the Harvard School of Public Health, Boston, Massachusetts.

films occurs to a significant extent at the normal functional residual capacity but, as the lungs are collapsed below the normal resting level, formation of such films increases. Even though on a quantitative basis the units closed by film formation may not represent a significant fraction of the lung volume, they may nevertheless be sufficient in number to account for the vesicular breath sounds (caused by breaking of the films) heard when a deep breath is taken. (*c*) The mechanical processes responsible for static hysteresis in the normal partially inflated lung thus include trapping, which makes a relatively high pressure necessary to reopen the units; hysteresis of the alveolar surface layer, which requires more energy to expand the surface than is recovered when the surface contracts; "geometrical" hysteresis, a difference in the elastic stresses when the lung unit is expanded, compared with deflation are the same volume range; as well as plastic behavior of lung structural elements. The quantitative significance of these various mechanisms remains uncertain, but it is likely that subsequent research in this area may provide important clues to the processes by which lung elasticity is lost in the development of emphysema.

INDICATIONS OF QUANTAL BEHAVIOR IN THE INFLATION AND DEFLATION OF RABBIT LUNGS[17]

LEON BERNSTEIN

The form of the pressure-volume curves of the lungs and thorax of the live, unanesthetized, curarized rabbit is known to be dependent on the recent history of inflation of the lungs. The first inflation to a volume greater than the tidal volume of the artificial ventilation used to keep the animal alive yields a curve which starts concave upwards, inflects abruptly into one of lower slope, i.e., increased compliance, and finally increases in slope once more; immediate reinflation provides a curve which begins with a lower slope than the first, but which increases in slope more rapidly and without the intermediate inflexion, so that the two curves end in the same pressure-volume point. Subsequent immediate reinflations provide curves identical, within experimental limits, with the first reinflation curve; but if reinflation is delayed, the curve is intermediate in form between the common form of the immediate reinflations and that of the earliest inflation, approaching the latter as the delay is increased. Deflation from any level of preinflation yields a curve of very constant form (hyperbolic and lying in the fourth quadrant) which lies consistently below any of the various inflation curves.

One hypothesis which has been advanced in explanation of these findings is that in normal tidal ventilation not all of the alveoli of the lungs are inflated; nor, indeed, are they all susceptible of inflation. For each alveolus there is a critical opening pressure which must be applied to it before it can open and so become susceptible of further inflation. In tidal ventilation, a certain population of alveoli—those having critical opening pressures lower than the peak value of the tracheopleural pressure gradient—are inflated; the first inflation to a volume larger than the tidal volume increases the tracheopleural gradient above the tidal peak value, and so causes additional alveoli to open. The deflation curve from any volume or pressure represents the truly elastic behavior of all those alveoli which have been inflated up to that volume or pressure; but the inflation curve represents the combination of two processes, elastic expansion and recruitment of alveoli, and so changes curvature at the point at which the second process starts. The difference between the deflation curve and the immediate reinflation curves is attributed to the closure of some of the newly recruited alveoli at the end of deflation, and the gradual transition in form of the delayed reinflation curves is attributed to a continuation of this process during a period of tidal ventilation.

Closure of recruited alveoli does not occur unless deflation proceeds to—or almost to—the resting expiratory level; moreover, it is not the length of a period of tidal ventilation enclosed between two large volume inflations, but the number of breaths included in that period, that determines the extent of alveolar closure between the inflations. Although these findings do not contradict the existence of critical closing pressures for alveoli, they suggest that other factors also are involved; the liability of an alveolus to closure probably depends on its deflation time constant, that is to say, on its compliance and on the conductance of its associated airway.

Discussion of Papers on Mechanical Properties of Alveoli

Dr. Mead helped to bring into focus the basic concept that the lung is potentially an "unstable" configuration because of physical laws governing the behavior of gas-liquid interfaces. This concept has three important implications: (*1*) The geometry of terminal units depends on their mechanical properties so that lung morphology and mechanics are inextricably bound together; (*2*) alveolar

[17] From the Cardiopulmonary Function Laboratory, Veterans Administration Hospital, Baltimore, Maryland.

structures are held in a delicately balanced state between collapse and overdistention not only by cellular tissue elements, but probably also by a uniquely variable (invisible?) surface-active material lining the alveolar walls (Clements' substance X); (*3*) certain pulmonary diseases may be caused by an excess (emphysema) or deficiency (infantile atelectasis) of this surface-active material, even though the ultimate manifestations of these diseases are clearly reflected in visible cellular changes.

The three papers on the mechanical properties of alveoli each contributed novel and provocative working hypotheses which, if supported by further evidence, may well demand fundamental changes in our concepts of the lung in health and disease. Dr. Clements' work with lung extracts suggested that pathologic increases of normally present surface-active material may ultimately prove to be responsible in part for such phenomena as air-trapping and the development of enlarged alveolar spaces—processes which have traditionally been believed to result directly from disturbances of the bronchopulmonary tissue itself. Dr. Radford demonstrated how intimately related the structure and mechanical behavior of alveolar tissue may be when he presented evidence that the former may be more dependent on the latter than *vice versa*. He also showed how the surface tension activity of the air-liquid interface could account for both the pressure-volume characteristics and the visible morphology of surface units of excised lungs. Finally, Dr. Bernstein built up a coherent conceptual picture of the mechanical behavior of the entire lung based on the thesis that each terminal unit responds to deformation in a characteristic way: each unit may be *recruited* at a different point on the inflation-deflation curves. This view involves not only the important concept of "normally nonfunctioning alveoli," but also the implication that statistical treatment of pulmonary phenomena may become a necessary part of any quantitative investigation of the functioning lung.

IV. RELATIONSHIPS BETWEEN ALVEOLAR STRUCTURE AND MECHANICAL FUNCTION

While this was the central issue of the conference, no one paper dealt specifically with the entire question. No formal paper dealt with the etiology of emphysema. Nevertheless certain fundamental concepts evolved from the free discussion which focused new light on both topics.

Perhaps the most unifying concept which emerged was that of the lung considered as an organ of connective tissue. Anesthesiologist Elwyn S. Brown (Buffalo, N. Y.) was impressed by the importance of the ground substance in maintaining the special relationships of the structures concerned in lung deformation. Biochemist Oscar Reiss (Denver, Colorado) felt that changes in pulmonary connective tissue might be induced by substances such as aminopterin. Dr. Hospelhorn felt that both Vitamin C and hormonal changes might alter pulmonary structure experimentally. Dr. Boren was keenly interested in the idea that injury to the ground substance might be initially responsible for the alveolar "holes" he demonstrated in emphysematous lungs. Dr. Pratt was impressed by the tremendous significance of surface tension in relation to the mechanical activities of the lung. Histochemist Gersh expressed the thinking of many of the participants when he asked: "What is the contribution of each of the various constituents of the alveolar wall to its elastic properties?"

The many-sided concept of alveolar stability was examined both theoretically and with quantitative measurements. It was clearly shown that the normal alveolar gas-liquid interface could stabilize alveolar tissue either because of its low surface tension or because of its capacity to increase surface tension with lung distention or both. Internist Howard Dayman (Buffalo, New York), however, pointed out the extent to which this theory depends on an assumed geometry of the terminal units; he thus emphasized the great need for a truly relevant anatomic model with which to interpret quantitative physiologic and biophysical data. Indeed, both Drs. Radford and Dayman indicated that, if the distending units are assumed not to be the alveoli themselves but the space around which they are grouped (Krahl's "corridor"), then a considerable reconciliation of divergent views becomes possible.

It was quite a departure from custom to hear the pathogenesis of emphysema considered without reference to obstruction to air flow. For instance, Dr. Pratt, using three-dimensional techniques, has satisfied himself that bronchial obstruction is not of importance in the initial stages of emphysema; he made the suggestion that the disease may be an irregularly progressive one in its own right and that air-flow obstruction may well be merely an incidental by-product of the disease process. Dr. Boren believes that he has found abnormal enlargement of the pores of Kohn; he feels that progression of emphysema consists in a "departitioning of space" which could result from localized areas of cellular death in alveolar walls with a concomitant loss of surface force. Parenthetically, it seemed that neither of these deductions could have been made from two-dimensional studies alone.

An important note of caution was introduced by the anatomic studies[18] of Dr. Richard McLaughlin (Clinical Investigation Center, U. S. Naval Hospital, Oakland, California). He showed the wide variations of the lung that exist in 7 mammalian species (monkey, cat, dog, cow, horse, pig, and sheep). The segmental anatomy, the distribution of the pulmonary and bronchial vasculature, the degree of shunting between the two systems, and their relationships to the airways differed to some degree in the 7 species; of the 7, the most similar to man was the horse, the only one having a natural condition ("the heaves") in any way resembling human emphysema. The difficulty with this observation is that equine "emphysema" is probably Farmer's lung in horses and due to the effects of molds or fungi. Those who are attempting to produce emphysema experimentally would do well to consider these rather surprising pulmonary differences between animal species.

The conference was well summarized by the following post-conference quotations:

Anatomist Vernon Krahl (Baltimore, Maryland): "I was most impressed by the vigorous and varied attacks which the physiologists are making upon the problems of pulmonary function. I could not help but marvel at the rather elaborate theoretical superstructures which they are willing and able to construct upon rather shaky anatomical foundations. However, I believe the morphologists went home from this conference with the realization that they have not kept pace with the physiologists."

Physiologist Solbert Permutt (Denver, Colorado): "The lungs must come to mean something more to the physiologist than bubbles and balloons, to the morphologist something more than cells, fibers and ground substance."

[18] Presented in part at the annual meeting of the American College of Chest Physicians, Atlantic City, New Jersey, June, 1959.

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Notes

SOIL SAMPLING IN AN URBAN FOCUS OF HISTOPLASMIN SENSITIVITY

School children living in the city of Dalton, in northwest Georgia, are more often infected with *Histoplasma capsulatum* than children living in the surrounding rural areas: 60 per cent compared with 20 per cent react to histoplasmin by the age of sixteen years.[1] Moreover, within the city, which has a population of 19,000 and covers a circular area approximately 3 miles in diameter, the rates are unusually high in one particular residential area[2] (Section A, figure 1). In that section nearly 25 per cent of the first- and second-grade children and more than 80 per cent of the high school students react to histoplasmin. In the rest of the city, reactor rates range from 10 per cent in first-grade children to around 50 per cent in high school students. The high prevalence area is located near the heart of the city and contains fine, well-kept old homes as well as modern, new ones, with spacious grounds. The lower prevalence area contains the textile mills surrounded by old frame houses crowded together and in poor repair and several small clusters of modern, though generally less pretentious, homes than those in Section A.

Since Emmons' original isolation of *H. capsulatum* from soil in 1949,[3] and the subsequent finding by Zeidberg and associates of its frequent occurrence in specimens collected in or near chicken habitats,[4] further studies by these and other investigators have established beyond question that *H. capsulatum* occurs naturally in soil, and particularly in soil enriched by excreta of chickens, bats, pigeons, and other birds.[5-10] The situation in Dalton seemed to offer an unusual opportunity to study possible other sources of *H. capsulatum* because of the sharp differences in histoplasmin sensitivity rates within a very small geographic area, the high rates being concentrated in a residential section singularly devoid of chickens, and lower rates in the rest of the city where chickens are not infrequent. The picture suggested that there might be one very potent focus or a scattering of foci of the fungus in Section A, the high prevalence area, and very few, if any, in the rest of the city. Moreover, an association of the fungus with chickens or other birds in this particular instance seemed quite unlikely. In an effort to find the source or sources of *H. capsulatum* in Dalton, soil specimens were collected for mycologic examination.

The first soil survey was done in April, 1958, in connection with a sanitary survey of the city, by a group of 20 sanitarians participating in a field training project under the direction of Richard Clapp of the Public Health Service Communicable Disease Center. Arrangements were made for the sanitarians to collect soil specimens from a prepared list of residences of elementary school children, selected at random from among those who had been skin tested in the school testing program the previous fall. From each of the specified residences, a specimen was to be collected from the southwest corner of the lot and from one or more of the following sites, if possible: under the house, in a dirt basement or storm cellar, chicken coops, flower garden, vegetable garden, compost pile, manure pile, barn, or barnyard. As all samples taken from a single premises were collected in a single container, the specimen for each address consisted of samples from one or several different sites on that particular premises. The samples were simply scooped into a half-pint, waxed, ice-cream container and covered with a waxed cardboard lid. A total of 311 specimens representing 311 residences of elementary school children were obtained during a two-day period.

[1] Edwards, P. Q., *et al.*: Dis. Chest, 1958, *34*, 467.

[2] Aronson, D. L., and Edwards, P. Q.: Am. Rev. Tuberc., 1959, *79*, 83.

[3] Emmons, C. W.: Pub. Health Rep., 1949, *64*, 892.

[4] Zeidberg, L. D., *et al.*: Am. J. Pub. Health, 1952, *42*, 930.

[5] Larsh, H. W., *et al.*: Am. J. Hyg., 1956, *63*, 18.

[6] Emmons, C. W.: Pub. Health Rep., 1958, *73*, 590.

[7] Ajello, L.: Am. J. Trop. Med. & Hyg., 1954, *3*, 897.

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[10] Hosty, T. S., *et al.*: Am. Rev. Tuberc., 1958, *78*, 576.

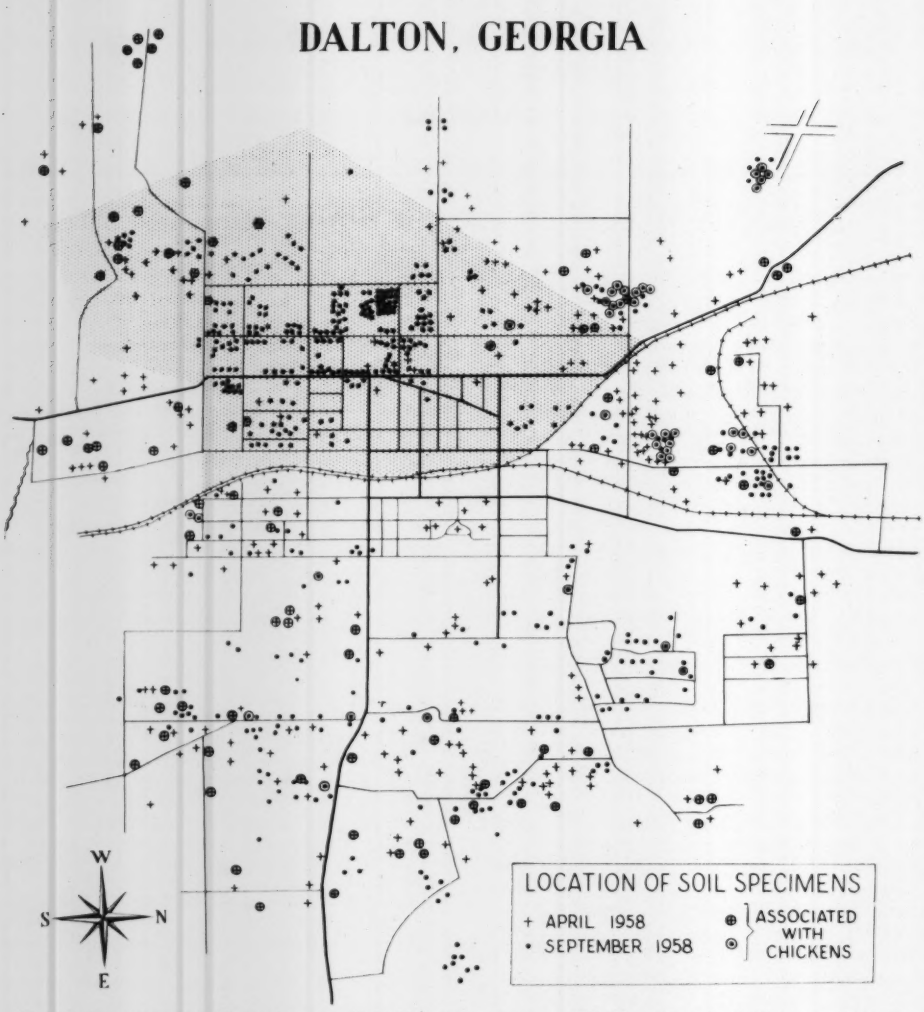

FIG. 1. Location of soil specimens collected in two surveys of Dalton, the shaded area on the map representing Section A and the unshaded area Section B.

In addition, 9 specimens were taken from the elementary school playgrounds and recreation parks. The locations of the 311 residences and 9 schools and parks are shown in figure 1; the sources of the specimens are listed in table 1. The relative scarcity of specimens from Section A, as well as from several other localities, was a chance result; fewer sanitarians participated in the survey than had been expected and, consequently, some groups of residences on the prepared lists were not visited.

After thorough mixing, a portion of each specimen was removed and pooled with corresponding portions of 5 or 6 other specimens collected in adjacent or nearby premises, making a total of 55 pooled specimens. These pooled specimens, as well as the 9 specimens from the schools and parks, were labeled only with serial numbers and taken to the Mycology Unit of the Communicable Disease Center for mycologic examination.

The laboratory procedure for examining the specimens has been described previously.[10] Briefly, the procedure was carried out as follows:

1. After thorough mixing, 1 heaping teaspoonful of soil was placed in 30 ml. of physi-

TABLE 1

NUMBER AND SOURCE OF SOIL SAMPLES COLLECTED IN TWO SURVEYS OF DALTON, GEORGIA

	April, 1958	September, 1958
Number of residence premises sampled in survey	311	242
Source of samples from residence premises		
Base of house, porch, steps	19	77
Under vent to attic	—	12
Under house, porch, steps; in basement	100	36
Shrubs, flower or vegetable garden	322	54
Base of tree	3	23
Open yard, children's play area, et cetera	248	74
In or beside drainage ditch	2	16
In or beside garage or shed	4	27
In or beside trash pile or garbage	2	16
In, under, or beside chicken coop	24	56
In, under, or beside dog pen	8	25
In, under, or beside cage for other animal	4	13
In compost or manure pile, barn, stable	37	6
Associated with pigeons or other birds	1	7
Total samples from residence premises	774	442
Total residence samples for individual examination	55*	442
Source of other samples		
Vacant lots	—	26
Woods	—	9
By stream or creek	—	3
Church, factory, public building	—	13
Schoolyard or recreation park	9	7
Abandoned silo	—	4
Miscellaneous (rotten tree stumps, gutters, excavations, cemetery)	—	15
Controls	—	9
Total number of samples examined mycologically	64	528

* Pooled from the 774 specimens collected on 311 residence premises.

ologic saline that contained 5,000 units of penicillin G and 1,000 units of streptomycin per ml.

2. After vigorous stirring, the soil was allowed to settle for approximately one hour.
3. Five milliliters of supernatant were then pipetted off and 1 ml. was injected intraperitoneally into each of 4 mice.
4. Eight weeks after inoculation, the mice were sacrificed, and small portions of their livers and spleens were inoculated into tubes of a neutral dextrose agar:

Neopeptone (Difco)	1.0 gm.
Dextrose	1.0 gm.
Chloramphenicol	0.005 mg.
Agar	2.0 gm.
Distilled water q.s.	100.0 ml.

(Adjusted to pH 7 before autoclaving at 120°C. for ten minutes.) Tissue from each mouse was inoculated into 4 tubes of this medium, 2 tubes with liver and 2 tubes with spleen tissue. A total of 16 tubes, therefore, were inoculated for each soil sample.

5. The tubes were incubated at 25°C. and examined periodically for the development of pathogenic fungi.

None of the 64 specimens was positive for *H. capsulatum*.

Because of the negative findings, and the thought that perhaps the soil specimens were collected at an unfavorable time of year, that they had not been taken from the right places, or that the pooling of specimens had reduced the chances of isolating the fungus, a second survey was undertaken in September, 1958, five months after the first survey. This time it was decided to concentrate, first, in Section A and then work in concentric circles around that section; and, instead of using a prepared list of randomly selected addresses, to follow hunches in choosing the "right" sites. The collecting was done by seven persons: the chief of the Mycology Unit of the Public Health Service Communicable Disease Center,

the director and two sanitarians of the Dalton-Whitfield District Health Department, and a statistician and two medical officers from the Research Section of the Public Health Service Tuberculosis Program. The group worked as two teams, each responsible for covering roughly half of the city.

Particular attention was paid to collecting specimens under eaves or air vents of houses and under trees, where bird droppings might be expected to have accumulated; in and around chicken coops, whether or not in use; in areas where children played, e.g., around swings, sandboxes, playhouses; and in or under animal pens or cages, whether or not in use. Residence property as well as public buildings, vacant lots, woods, lumber yards, excavations, streets, and a cemetery were included. The specimens were collected by scooping up soil into 6-ounce, glass, screw-top jars. Altogether 519 samples, each from a single site, were collected during a two-day period. The sites from which the 519 specimens were taken, are shown in figure 1 and table 1, 242 of which were from private residence premises.

The specimens, which were not pooled in the second survey, were labeled with random numbers and taken to the Mycology Unit of the Communicable Disease Center. As it was deemed necessary by two of the writers (P. E. and D. A.) to control the laboratory procedure, a soil sample from which *H. capsulatum* had been recovered several years previously was obtained from Miss Charlotte Campbell of the Walter Reed Army Institute of Research. This sample (identified here as Campbell #4) was divided into 10 portions, each of which was placed in a separate jar labeled with a random number. To 5 of these samples were added approximately equivalent amounts of soil removed from 5 different Dalton specimens. These are referred to as the "mixed controls." Nothing was added to the other 5, referred to as the "unmixed controls." One of the unmixed controls was taken back to Miss Campbell for retesting: she reported later that she had failed to recover *H. capsulatum*. The remaining 9 (5 mixed and 4 unmixed) were included with the 519 Dalton soil specimens, thus giving a total of 528 specimens for mycologic examination. The identity and total number of control specimens included in the study were not made known to the Mycology Unit.

The procedure for mycologic examination of the specimens was the same as for the earlier survey.

Two of the 528 samples yielded *H. capsulatum*. One was a sample which had been collected from the north side of a house located in Section B, about half a mile from the edge of Section A. This represents the first isolation of *H. capsulatum* from Georgia soil, although the fungus has been isolated many times from wild animals in the southern part of the state.[11, 12]

The other sample which was positive for *H. capsulatum* was one of the Campbell #4 soils mixed with a sample of Dalton soil. Because *H. capsulatum* was not recovered from the 8 other control specimens, it was decided to retest all 9 of them as well as the one Dalton soil from which *H. capsulatum* had been recovered. It was also decided to submit aliquots of those 10 specimens to Dr. Chester Emmons of the National Institutes of Health and to Miss Campbell for mycologic examination.[13] Since one of the unmixed control soil specimens had been used up in the initial examination, only 9 soil specimens were re-examined: the 5 mixed controls, 3 unmixed controls, and the one Dalton soil which was positive for *H. capsulatum*. The results obtained by all three laboratories were identical: the 2 specimens which were positive on the initial examination were again positive and, in addition, *H. capsulatum* was recovered from 2 more of the mixed control specimens.

Since the unmixed controls were again negative on retesting (suggesting that the Campbell #4 specimen seemed to have deteriorated in storage) and since the 3 positive controls had been mixed with Dalton soils, the question arose as to the source of the *H. capsulatum* in these specimens. The possibility existed that the Dalton soil components of the mixed specimens may have harbored the fungus, even though the corresponding Dalton soils which provided the Dalton components were negative on the initial examination. Accordingly, retests were made on those 3 Dalton soil samples. They again failed to yield *H. capsulatum*. In the opinion of one of the writers (L. A.), this attempt to use a control in the soil isolation work revealed the difficulties inherent in the selection and preparation of controls for a study of this type.

In July, 1959, ten months after the second survey, a visit was made to the premises in Section B from which the single soil specimen yielding *H. capsulatum* had been collected. Ten more samples were obtained from various sites around the house, particular efforts being made to select specimens that might be considered fairly representative of the area from which the original positive specimen had been obtained. The specimens were examined in the Mycology Unit at the Communicable Disease Center, employing the same procedure as previously. None of the 10 specimens yielded *H. capsulatum*.

[11] EMMONS, C. W., *et al.*: Pub. Health Rep., 1949, *64*, 1423.

[12] AJELLO, L. (unpublished).

[13] Dr. Ajello acknowledges the cooperation of Dr. Emmons and Miss Campbell in testing these specimens.

Essentially negative results from soil specimens collected from approximately 1,300 different sites throughout the city of Dalton certainly were not expected. The unusually high prevalence of skin sensitivity to histoplasmin in school children, its association with pulmonary calcification in nonreactors to tuberculin,[14] and the fact that several cases of clinically active histoplasmosis have been diagnosed in recent years, all point to indigenous sources of *H. capsulatum* within the city. The fungus would seem not only to be present but readily accessible to large numbers of persons, particularly those living in Section A, where the chance of becoming infected by the age of sixteen years approaches 90 per cent.

It may be that only small numbers of organisms are in the Dalton soil; that a very small infecting dose is sufficient to produce sensitivity to histoplasmin in human beings; and that the present method of examining soil for *H. capsulatum* is not effective unless the soil is heavily contaminated with the fungus. Although it has been shown that implantation of single spores generally causes infection in mice,[15] the flotation method is relatively inefficient for extracting spores from soil.[16, 17] Such a situation could account for the present findings.

The essentially negative results reported here thus raise the question whether the principal source of histoplasmal infection for a high proportion of the healthy population in Dalton, and perhaps in other areas as well, may not be soil but elsewhere in the environment. Many of the reports in the literature in which the fungus has been successfully isolated from soil have been in connection with isolated cases of the disease or localized outbreaks in small groups of persons with common exposure in a closed environment, such as a bat-infested cave or an unusually dusty task, such as tearing down an old chicken coop. The present study suggests that the epidemiology of benign and subclinical infection with *H. capsulatum*, as evidenced only by the presence of histoplasmin sensitivity in large numbers of healthy persons, may well involve hitherto unrecognized sources of the fungus—a possibility being further investigated in Dalton.

In summary: Attempts to isolate *H. capsulatum* from soil collected throughout the city of Dalton, Georgia, were successful in one of nearly 600 samples. This is the first time that the fungus has been recovered from Georgia soil, although it has been found previously in wild animals in the southern part of the state. Epidemiologic studies of skin sensitivity to histoplasmin in residents of Dalton and the surrounding areas indicate that histoplasmal infection is highly prevalent in one section of the city. Failure to isolate *H. capsulatum* from any samples of soil collected in that particular section suggests that further search for sources of the fungus might be made elsewhere in the local environment.

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THE PATHOGENICITY FOR GUINEA PIGS OF ISONIAZID-RESISTANT TUBERCLE BACILLI ISOLATED FROM PATIENTS ON A HIGH-DOSE ISONIAZID REGIMEN

The following presentation constitutes an experience with isoniazid-resistant tubercle bacilli of diminished catalase activity excreted by patients with pulmonary tuberculosis who had been treated on a daily regimen of 800 mg. of isoniazid and 100 mg. of pyridoxine. The results of animal inoculation experiments with these strains represent a sampling of strains from 45 patients with chronic, predominantly far advanced tuberculosis.

Direct isoniazid-susceptibility, catalase, and guinea pig tests on monthly sputum specimens were performed essentially as described in the *Tuberculosis Laboratory Methods* (1955 and 1956) of the Veterans Administration. The bacteriologic data were supplemented by monthly serum protein fractionations which were made with the Spinco[1] Model R paper electrophoresis apparatus and methods.

A total of 51 pairs of guinea pigs were inoculated to test the pathogenicity of certain strains isolated from the patients. Two pairs of animals were inoculated for each monthly specimen being tested: one pair from a control culture and another from a culture containing an isoniazid concentration of 10 γ per ml. This was done to reveal any susceptible organisms that may have been obscured in the control culture, which contained no isoniazid. However, in view of the fact that the results from the two sets of cultures so closely approximated each other, no distinction between the two was made in the accompanying tables. An obvious exception to this statement appears in the data for *A.A.C.* (table 1). In this case a great difference in catalase activity was reflected in the results observed in the guinea pigs.

The first set of studies consisted of 28 pairs of guinea pigs which were inoculated almost entirely with isoniazid-resistant, catalase-negative organisms (table 1). In this study, a strain was considered resistant when its growth in an isoniazid concentration of 5 γ per ml. tube was equivalent to that in the control culture. Four pairs of inocula were "borderline resistant," i.e., resistant to 1 γ per ml. and susceptible to 5 γ per ml., and one of these pairs was catalase positive.

The pigs were sacrificed at various intervals between six and twenty weeks. It was hoped thereby to reveal any delayed infections that might possibly result from such strains of tubercle bacilli. As may be seen in table 1, most of these strains of tubercle bacilli produced either no disease or an attenuated form of disease. By contrast, however, one strain from a patient with fibrotic cavitary tuberculosis did prove to be "moderately" pathogenic, and a strain from a patient with cavitary exudative tuberculosis induced definite disease in the guinea pigs. Animals inoculated with the last-named 2 strains comprised 7 (12.5 per cent) of the pigs in this group. Moreover, remnants of tuberculous disease, particularly in the spleen, were frequently evident on gross examination. The death of the patient listed as *E.J.H.* in the table will be commented on below.

In view of this evidence of pathogenicity and the observation of Stief[2] that earlier sacrifice of the guinea pigs (twenty-eight days) may reveal disease that might subsequently be obscured due to regression, the approach indicated in table 2 was adopted.

In this second group of studies, 11 pairs of guinea pigs were inoculated with isoniazid-resistant, catalase-negative organisms. Ninety-one per cent (10 of 11 animals), 100 per cent (7 of 7 animals), and 25 per cent (1 of 4 animals) sacrificed at about four, six, and twenty-two weeks, respectively, exhibited tuberculous disease. This represented a total of 18 of the 22 pigs employed. Ten of 11 guinea pigs showed some degree of regression of disease at six and twenty-two weeks; however, disease was still present in 8 of these animals.

The data on *A.E.H.* and *A.B.* would seem to indicate that the patients with fibrotic cavitary disease who had normal electrophoretic serum protein patterns and "unchanged" clinical conditions might merely have attained some sort of equilibrium with a still potentially pathogenic organism. While the death of patient *A.B.* was ascribed to right heart failure (cor pulmonale), 3 of these patients did succumb to tuberculosis a year or more after the presently reported observations. The electrophoretic patterns of 2 patients (*W.S.* and *E.J.H.*) reflected the presence of tissue destructive processes, although the patients harbored drug-resistant and catalase-negative organisms which induced either a regressive or attenuated pattern of disease in the guinea pig. However, the third patient (*J.B.*), whose animal results were unfortunately invalid and not included, presented a normal electrophoretic serum protein pattern.

The patient *M.J.C.* belonged to a group of

[1] Spinco Division, Beckman Instruments, Inc., Belmont, California.

[2] Stief, M. E.: Am. J. Med. Tech., 1956, *22*, 265.

TABLE 1

GUINEA PIGS SACRIFICED BEYOND SIX WEEKS

Patient	N.A.	S.V.	J.B.T.	E.J.H.	C.D.	A.B.D.	A.A.C.
Guinea pig pairs inoculated	2	6	6	2	8	2	2
Isoniazid-susceptibility	Resistance to 10 γ	R10	R10	R10	R10	R1, S5	R1, S5
Catalase reaction*	0	0	0	0	0	C:1+† 10:0	C:3+ 10:tr
Virulence of inocula‡	0	0 to 2+	0	1+ to 1½+	0 to 4+	C:1½+ 10:1+	C:4+ 10:1½+
Clinical appraisal of patient	Fibrotic, cavitary tuberculosis, unchanged	Fibrotic, cavitary tuberculosis, unchanged	Fibrotic, cavitary tuberculosis, unchanged	Fibrotic, cavitary tuberculosis, unchanged (died)	Exudative, cavitary tuberculosis, worse	Fibrotic, cavitary tuberculosis, unchanged	Fibrotic, cavitary tuberculosis, unchanged

* 0 = negative; tr. = trace; 1+ = little; 2+ = diminished but moderate; 3+ = large
† Refers to culture tube from which inoculum was made; C = control, 10 = 10 γ
‡ 0 = Avirulent (negative cultures)
1+ = Attenuated (cultures recovered from lymph nodes only)
2+ = "Moderately" virulent (cultures recovered from spleen and liver or lungs also)
3+ = Virulent (cultures recovered from all tissues, but to lesser degree than 4+)
4+ = Virulent (cultures recovered from all tissues, to extent of 50 or more colonies)

TABLE 2

GUINEA PIGS SACRIFICED AT ABOUT FOUR, SIX, AND TWENTY-TWO WEEKS

(All Inocula Isoniazid-resistant and Catalase-negative)

Patient	A.E.H.		A.B.		W.S.			M.J.C.*		J.E.B.		C.W.C.		E.M.	
Guinea Pig pairs inoculated	2		1		2			1		2		1		2	
Time of sacrifice (days)	31	43	28	43	32	42	154	28	45	30	44	35	154	35	154
Virulence of Inocula†	3+	2+	2½+	2+	2½+	2½+	1+	3½+	2½+	3+	2½+	3½+	2+	2½+	1+
Clinical appraisal of patient	Fibrotic, cavitary tuberculosis, unchanged		Fibrotic, cavitary tuberculosis, unchanged (died of heart disease)		Fibrotic, cavitary tuberculosis, unchanged (died)			Fibrotic, cavitary tuberculosis, improved		Exudative, cavitary tuberculosis, worse (died)		Exudative, cavitary tuberculosis, worse (died)		Exudative, fibrotic, cavitary tuberculosis, worse (died)	

* Previously untreated patient
† Refer to pathogenicity scale under table 1

patients with recently active and previously untreated tuberculosis that generally responded well to treatment. The potential pathogenicity of these tubercle bacilli is apparent from the clinical deterioration and subsequent deaths of the patients with cavitary exudative disease listed in table 2.

A third group of 12 pairs of guinea pigs were inoculated with drug-susceptible and drug-resistant strains from 6 patients who produced ba-

TABLE 3

GUINEA PIG DATA ON PATIENTS WHO PRODUCED TUBERCLE BACILLI WHICH CONVERTED FROM ISONIAZID-SUSCEPTIBLE TO ISONIAZID-RESISTANT

Patient	Y.B.				S.F.F.				S.S.*				R.P.*				J.W.*				B.K.*			
Guinea pig pairs inoculated	1		1		1		1		1		1		1		1		1		1		1		1	
Isoniazid susceptibility	Susceptible to 0.1 γ/ml.		Resistant to 10 γ/ml.		S0.1		R10		S0.1		R10		S0.1		R10		S0.1		R10		S0.1		R10	
Catalase reaction†	3+		3+		3+		3+		3+		1+		3+		3+		3+		3+		3+		1+	
Time of sacrifice (days)	31	41	27	46	27	46	Died 22	46	28	41	28	49	28	43	28	43	30	Died 33	27	46	Died 26	49	28	42
Virulence of inocula‡	2+	2+	0§	0§	2½+	3½+	4+	4+	4+	4+	1½+	1½+	2½+	3½+	3½+	2½+	4+	4+	2+	2+	2+	3½+	2+	3+
Clinical appraisal of patient	Fibrotic, cavitary tuberculosis, unchanged				No cavity, fibrotic tuberculosis, unchanged				No cavity, fibrotic exudative tuberculosis, improved				Cavitary, exudative tuberculosis, improved				Cavitary, exudative tuberculosis, improved				Cavitary, exudative tuberculosis, improved			

* Previously untreated patient.

† 0 = negative; 1+ = little; 2+ = diminished but moderate; 3+ = large.

‡ Refer to virulence scale under table 1.

§ Subculture made at time of inoculation of guinea pigs yielded no growth (*see* text).

TABLE 4

INCONSISTENT PATTERNS OF CATALASE PRODUCTION[1] BY TUBERCLE BACILLI EXCRETED BY SOME PATIENTS[2]

Month	A.D.B.				A.A.C.				B.W.B.				R.P.				B.K.				S.S.			
	Control		10γ/ml.		Control		10γ/ml.		Control		10 γ/ml.		Control		10 γ/ml.		Control		10γ/ml.		Control		10γ/ml.	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
1	tr	0	0	0	0		3+[4]																	
							tr[4]	1+	0		1+		3+[3]	3+	1+[4]	1+[4]					3+	3+		
2	1+	0			0		0		1+		0		1+	3+	0	0	3+	3+[3]			3+[3]	3+		
															1+	1+								
3	1+	0	0	tr	0		0		0		tr		1+	1+	1+	1+	2+		2+	1+	2+		1+[4]	1+[4]
				1+													1+							
4	0	tr	0	0	0	3+	tr		0			3+	2+	2+	1+	1+		tr	tr	1+	1+[3,6]		1+[4]	1+[4]
5	1+[3]	tr	0	0[3]	tr		tr						3+[3,5]	3+[6]	0	0	2+	tr	tr	tr	1+			
							1+								tr	tr								
6	1+	tr	0	tr	3+[3]			tr[3]	1+			3+	1+	2+	tr	tr	1+	1+[3]	1+	2+				
														1+			2+							
7	1+		3+		0		0						3+	3+	1+	tr		2+	1+	1+				
8		0		0	0		0			1+	3+	3+	3+	3+	1+	1+								
9	3+		1 γ	1 γ	0	0	3+		1+	1+	3+	3+[4]	3+	1+	tr	1+								
			tr	tr						2+			2+											
10	3+			3+	3+		0																	
11	3+		3+		3+		2+																	
12	3+		3+		0		2+[4]																	
			1+																					

[1] Catalase production: 0 = negative; tr = trace; 1+ = little; 2+ = diminished but moderate; 3+ = large.

[2] Many catalase tests on an individual tube were performed two or three times, but this is only indicated above in those instances in which the results were different.

[3] Specimens from which guinea pig inocula were made.

[4] Catalase test performed on a single colony.

[5] Catalase test performed on a subculture.

[6] Catalase test performed on a culture from the superficial inguinal lymph nodes.

cilli which converted from drug susceptible to drug resistant in the course of treatment (table 3). In these instances, the guinea pigs were inoculated only with bacilli from the control cultures. Diminished catalase activity accompanied isoniazid resistance only in the resistant strains produced by *B.K.* and *S.S.* Neither of these strains produced a regressive pattern of disease in the guinea pig, but the resistant bacilli from *S.S.* produced definitely less disease than was produced by the susceptible bacilli previously isolated from the same patient. This again represents a failure to find any absolute correlation between pathogenicity for guinea pigs and the catalase activity of these resistant organisms. Subsequently, tubercle bacilli disappeared from the sputum of patients *J.W.* and *S.S.* bacteriologically.

The other patients continued to discharge tubercle bacilli, and the other resistant strains remained catalase positive. The results obtained with these strains are therefore included for completeness of the presentation. It should be noted that 4 of these strains were from previously untreated patients with recently active disease who showed clinical improvement and normal electrophoretic serum protein patterns. The isoniazid-resistant culture from *Y.B.* would not grow on subculture and produced no disease in the guinea pig. Nevertheless, the data on this patient were included to illustrate that what has been designated as "moderate" disease can occasionally be induced in the guinea pig by an isoniazid-susceptible catalase-positive strain, and that such disease may be more significant than might be inferred from the arbitrary pathogenicity scale.

In table 4 the catalase production of tubercle bacilli isolated at monthly intervals from 6 patients on the high-isoniazid regimen is depicted. The inconsistent patterns exhibited by these strains is evidence of a further complication that may be introduced into the question of whether the patient is harboring a pathogenic strain. Three patients (*A.D.B.*, *A.A.C.*, and *B.W.B.*) had strains that were "borderline resistant" in terms of isoniazid. For about half the period of observation, *A.D.B.* produced organisms with little or no catalase, but subsequently bacilli with vigorous catalase activity appeared. Tubercle bacilli exhibiting negative and high catalase reactions were intermittently isolated from *A.A.C.*, and the cultures from *B.W.B.* grown in isoniazid concentrations yielded vigorous catalase producers in four monthly specimens, while the control cultures yielded deficient or no catalase producers. Of the 3 patients in whom isoniazid-resistant tubercle bacilli emerged to predominance, the strains from *B.K* and *S.S* showed a change from vigorous to diminished catalase activity. Such was not the case with *R.P.*, who intermittently produced high and low catalase samples. The question therefore arises whether it is possible to generalize about the pathogenicity of the tubercle bacilli present in such cases or whether the pathogenicity that is determined actually holds only for the particular organisms isolated from a particular specimen. The variation in the catalase activity of tubercle bacilli from one culture tube to another in the replicate isoniazid-susceptibility culture tubes and even within the same tube, which is evident in table 4, was also made apparent by the technique employed in this study. Several colonies were transferred to the bottom of a Kahn tube and covered with catalase reagent. Variation in catalase activity could be observed from one clump of bacteria to another with the aid of a magnifier (4×). Therefore, the heterogeneity of a specimen in this respect may be obscured by other techniques and equated with the gas evolution of the most active bacilli in the sample.

In summary: Fifty-one pairs of guinea pigs were inoculated to test the pathogenicity of isoniazid-resistant and catalase-deficient strains of tubercle bacilli isolated from patients on a high-dose isoniazid regimen. No clear-cut or absolute correlation of virulence with catalase activity was observed in these cases. Sufficient evidence has been encountered to suggest that generalizations with respect to this problem be viewed circumspectly and that such patients be approached on an individual basis, giving due consideration to all the relevant data.

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July 14, 1959

A MICROMETHOD FOR THE ROUTINE DIFFERENTIATION OF HUMAN TUBERCLE BACILLI FROM OTHER MYCOBACTERIA IN PRIMARY CULTURES[1]

On the basis of Pope and Smith's experiments,[2] it has become known that tubercle bacilli of human origin contain much more nicotinic acid than other strains of mycobacteria, and the nicotinic acid is present in the culture medium in which the bacilli are grown.

Konno[3] developed a very good method for distinguishing human strains with the help of the sensitive colorimetric reaction discovered by König[4] and used by him for identification of pyridine compounds. Konno and his co-workers[5] showed that his procedure is suitable for the differentiation not only of bovine strains, but also of the many unclassified strains of mycobacteria which are of such general interest today. The identification of the unclassified mycobacteria has become important not only for the pathologist but also for the clinician.

The practical use of the "direct chemical method" described by Konno for routine purposes presents difficulties because it requires a relatively large amount—at least 20 mg.—of bacteria.[6-8]

The present writer has developed a method by the use of which even a small quantity of bacteria, about 1 mg., can be examined on routine cultures. More than a few milligrams of bacteria seldom grow on the primary Löwenstein-Jensen and other solid media. The method described below may be used directly without inoculation. This is extraordinarily important for gaining time as well as because it was found at the Diagnostic Laboratory of the Institute that many cultures do not grow long in subcultures. Consequently, their identification as strains deviating from the normal becomes impossible.

The same problem was found in the studies of Konno and his co-workers[5] in which 85 of 663 routine cultures examined did not grow in subcultures, and so differentiation could not be done.

The present writer has found that benzidine is more suitable than aniline for the purpose of the qualitative testing of nicotinic acid. This reagent gives a violet-pink compound with bromine cyanide and nicotinic acid which stains the precipitating benzidine crystals. The color contrasts well with the colors of most fluid and solid media. Moreover, the color produced by chromogenic bacteria does not disturb the reaction. Furthermore, the sensitivity of the test using benzidine is fourfold that of the aniline reagent. For the direct chemical testing of a small quantity of bacteria, the value of the procedure is evident.

For the micromethod the following simple equipment is to be used:

Thin conic tubes with 55 to 65° angles, and 16 mm. in diameter used for centrifuging of urine sediment.

Pasteur pipettes that deliver a drop of 9 to 12 mg. from the reagents and other pipettes giving 18 to 22 mg.

A thicker platinum wire in loop-holder. The end of the wire is flattened out to provide a microspatula for taking out the bacteria.[9] Thin glass rods, rounded.

A 3 per cent alcoholic solution of benzidine (freshly crystallized reagent grade substance). The solution is stable for weeks if kept in a brown glass at 0 to 4°C. The ethanol should be absolutely free of pyridine compounds.

Concentrated watery solution of bromine cyanide (about 10 per cent) which can be used for several months if kept in a refrigerator.

Procedure for routine cultures on solid medium (Löwenstein-Jensen): For the examination of about 1 mg. of bacteria, a benzidine solution of 9 to 12 mg. is delivered by Pasteur pipette to the bottom of a conic tube. The bacteria are added with the microspatula to the fluid, and the suspension is homogenized with a thin glass rod. Then the fine granules clinging to the side of the tube will be pushed with the microspatula into the liquid. After this the suspension is dropped into a bromine cyanide solution of about the same weight. The bacteria will become slightly pink in color and the benzidine crystals, violet-pink, at once or in ten minutes. The tubes are examined with a magnifying glass in reflected daylight. The more

[1] This micromethod was presented in a lecture at the Congress of the Hungarian Microbiological Society, November 11, 1958.

[2] Pope, H., and Smith, D. T.: Am. Rev. Tuberc., 1946, *54*, 559.

[3] Konno, K.: Science, 1956, *124*, 985; Beitr. Klin. Tuberk., 1958, *119*, 1.

[4] König, W.: J. prakt. Chem., 1904, *69*, 105; Chem. Berl., 1954, *87*, 825.

[5] Konno, K., *et al.*: Am. Rev. Tuberc., 1958, *77*, 669.

[6] Bonicke, R.: Jahresbericht des Tuberkuloseinstituts Borstel, 1956–1957.

[7] Marek, A.: Beitr. Klin. Tuberc., 1957, *117*, 529.

[8] Gilani, S., and Selkon, J. B.: Tubercle, 1958, *39*, 396.

[9] It is necessary to practice taking out a suitable quantity of bacteria from the medium, for which purpose an analytic scale may be used.

nicotinic acid there is in the bacteria, the sooner the crystals become colored and the more intensive their color will be. For the testing of a bacterial mass of about 2 to 3 mg., 18 to 22 mg. from the reagent solutions are employed.[10]

For a whole series of tests the following controls are set up:

Reagent control: One drop of benzidine solution and one drop of bromine cyanide, which must remain colorless for at least ten minutes.

Bacteria control: About 1 to 3 mg. of bacteria from a bovine strain, aged thirty-five to fifty days, recently cultivated on Löwenstein-Jensen medium,[11] are tested against the reagents in concentrations of 9 to 12 mg. and 18 to 22 mg. For the checking of the activity and coloring intensity of the reagents, it is advisable to use a similar control with a human strain, recently cultivated on Löwenstein-Jensen medium.

It should be noted that the nicotinic acid contents of the cultures of tubercle bacilli grown on Löwenstein-Jensen medium are not always the same in different parts of the tube. The populations grown in the water of condensation and above it often contain more nicotinic acid than those grown higher in the tube. The condensed water itself can be used also for the test if the bacterium has grown on a larger surface. Even the whole culture could be used immediately for the testing with 0.5 to 0.05 ml. reagent.[7] The Löwenstein-Jensen routine cultures are suitable for examination four to five weeks after the growing has started.[8]

As mentioned for the purpose of a "direct chemical method," benzidine is generally much more suitable than aniline as it makes a more striking differentiation possible. When testing liquid media, one part of the medium, without filtration, is used with equal volumes of benzidine and of bromine cyanide solution. One microdrop usually suffices for the examination.

The procedure has been employed with 5 saprophytic strains of mycobacteria: *M. butyricum*, *M. Pellegrini*, *M. Frieburg*, and *M. phlei;* with 4 laboratory bovine strains; with 2 BCG strains; and with 2 avian strains. The test yielded negative reactions (sometimes weakly positive) in all instances. By contrast, 8 laboratory strains of human origin all gave very strong reactions. Three isoniazid-resistant and streptomycin-resistant strains gave negative reactions. An additional 247 cultures of mycobacteria isolated routinely in the laboratory were tested, and 5 gave negative reactions. Ninety-four cultures grown on Sula medium were studied, and 2 gave negative reactions. In the last-named tests, the tubes were placed in a boiling water bath for fifteen minutes before testing without influencing the outcome of the test.

In summary: With the use of benzidine and bromine cyanide, a micromethod for determining the niacin content of mycobacteria has been developed. Only a small quantity (usually only 1 mg.) of bacterial cells are necessary for performance of the test.

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July 30, 1959

[10] In certain cases aniline can also be used with this method. For 2 to 3 mg. of bacteria, 9 to 12 mg. of aniline solution are added (4 per cent in alcohol) and after homogenization the same weight of bromine cyanide is added. The suspension must be centrifuged immediately and care taken to ensure that the tube is securely closed with a rubber plug. The color of the suspension is then examined.

[11] It was found that aged laboratory strains sustained on fluid media (Sula, Sauton) produced much more nicotinic acid than recently isolated strains or strains transplanted continuously on Löwenstein-Jensen medium.

[12] The writer wishes to express his gratitude to Dr. J. Szabo and Dr. N. Kertai for making available the strains and routine cultures necessary for the above experiments.

THE USE OF *p*-NITRO-α-ACETYLAMINO-β-HYDROXY-PROPIOPHENONE (NAP) IN THE DIFFERENTIATION OF MYCOBACTERIA

Attempts to differentiate mycobacteria on the basis of their susceptibility to chemical agents were first initiated by Cattaneo and associates.[1] They demonstrated that tubercle bacilli of both human and bovine strains were inhibited by 1 γ of PAS per ml., whereas avian strains and the unclassified mycobacteria were resistant to at least 100 γ per ml. The use of PAS in treatment, however, has resulted in an increased number of PAS-resistant strains so that the usefulness of this method is limited.

The possibility of using the spectrum of resistance to a number of antimicrobial drugs for this purpose has also been studied.[2-7] Meissner[5] differentiated between unclassified mycobacteria and typical pathogenic strains by systematically determining their resistance to isoniazid, PAS, streptomycin, and amithiozone.

Bonicke[8, 9] recommended theonyl-2-hydrazine and furfuroyl-2-hydrazine to differentiate human from bovine strains. The bovine strains were susceptible to these compounds in a concentration of 1 γ per ml., whereas the human strains grew in concentrations of the drug up to 25 γ per ml.

Gastambide-Odier and Smith[10] studied thirty-five different strains of mycobacteria, including human, bovine, and avian strains as well as saprophytic and unclassified strains. The human, bovine, saprophytic, and *M. fortuitum* strains were inhibited by concentrations of 1:40,000 of neotetrazolium, whereas the unclassified and the avian strains were not.

The present report deals with studies of this question using *p*-nitro-α-acetylamino-β-hydroxy-propiophenone (NAP), an intermediate compound prepared during the synthesis of chloramphenicol. In a previous report,[11] this compound was shown to have four times the antituberculous activity of chloramphenicol.

Medium: A modified Sauton medium[12] was used. This was dispensed into 20- × 175-mm. test tubes, 9 ml. per tube.

Dilutions of p-nitro-α-acetylamino-β-hydroxy-propiophenone (NAP): A basic solution of this compound in a concentration of 6 mg. per ml. was prepared by adding the required amount of NAP to a known quantity of ethyl alcohol in an Erlenmeyer flask and swirling in a hot water bath (65°C.) until dissolved. Serial dilutions were then made in sterile distilled water. One milliliter of each dilution was added to the Sauton medium so that the final concentrations of compound in a series of tubes were 2.5, 5.0, 10, 20, 40, 80, and 160 γ per ml. A control tube without NAP was used in each series.

TABLE 1

SUSCEPTIBILITY OF DIFFERENT MYCOBACTERIAL STRAINS TO *p*-NITRO-ACETYLAMINO-HYDROXY-PROPIOPHENONE (NAP)

Strains	Number of Strains Studied	Number of Strains Showing Growth in NAP						
		160	80	40	20	10	5	2.5
Human	42	0	0	0	0	0	24	42
Bovine	12	0	0	0	0	0	6	12
Avian	12	0	4	11	12	12	12	12
Chromogenic	61	1	34	57	61	61	61	61
Saprophytic	8	5	6	8	8	8	8	8

Inoculum: Using a pipette which delivered 0.05 ml. per drop, each tube was inoculated with three drops of ten- to fourteen-day-old cultures grown in Dubos Tween®-albumin medium, and then incubated at 37°C. for fourteen days and examined for growth.

Forty-two human, 12 bovine, 12 avian, 61 chromogenic, and 8 saprophytic strains were treated as above. The results are shown in table 1.

By applying this technique to these 135 strains

[1] CATTANEO, C. M., *et al.*: Lotta contro tuberc., 1950, *20*, 291.

[2] YOUMANS, G. P.: Bull. Union internat. contre tuberc., 1958, *28*, 128.

[3] WOLINSKY, E., *et al.*: Am. Rev. Tuberc., 1957, *75*, 180.

[4] MAYER, R. L., *et al.*: Am. Rev. Tuberc., 1958, *77*, 694.

[5] MEISSNER, G.: Bull. Union internat. contre tuberc., 1958, *28*, 152.

[6] TARSHIS, M. S., *et al.*: Am. Rev. Tuberc., 1955, *72*, 119.

[7] ROGUL, M., *et al.*: Am. Rev. Tuberc., 1957, *76*, 697.

[8] BONICKE, R.: Ztschr. Hyg., 1958, *145*, 263.

[9] BONICKE, R.: Naturwissenschaften, 1958, *45*, 292.

[10] GASTAMBIDE-ODIER, M., AND SMITH, D. W.: Am. Rev. Tuberc., 1958, 77, 662.

[11] EIDUS, L., *et al.*: Antibiotics & Chemother., 1959, *9*, 491.

[12] EIDUS, L., *et al.*: Canad. J. Pub. Health, 1958, *49*, 18.

of mycobacteria, it was found that all of the 54 human and bovine strains studied were susceptible to *p*-nitro-α-acetylamino-β-hydroxy-propiophenone (NAP) at a concentration of 10 γ per ml. The remaining 81 strains, including the avian, chromogenic, and saprophytic strains, were inhibited only by concentrations of 40 γ or more per ml. This simple, easily reproducible method for determining the susceptibilities of mycobacteria to NAP may be useful in diagnostic laboratories as a screening test for differentiating human and bovine strains from other types of mycobacteria. The differentiation between human and bovine strains may be made by determining the niacin production of different strains, using adaptations of Konno's[13, 14] method, or by testing the susceptibility to hydrazine derivatives as outlined by Bonicke.[8, 9] In veterinary medicine, the NAP test may be used along with other methods for the rapid cultural differentiation of avian and bovine strains. The fact that NAP is not used clinically enhances its value for diagnostic purposes.

In summary: Human and bovine strains of *M. tuberculosis* are susceptible *in vitro* to relatively low concentrations of *p*-nitro-α-acetylamino-β-hydroxy-propiophenone (NAP). Avian strains and various strains of unclassified mycobacteria are considerably less susceptible to NAP, so that the susceptibility to this compound can be used to distinguish between these two broad groups of mycobacteria.

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November 16, 1959

[13] Konno, K., *et al.*: Am. Rev. Tuberc., 1958, 77, 669; 675.

[14] Eidus, L., *et al.*: The production of niacin by mycobacteria, presented at the 27th annual meeting of the Laboratory Section of the Canadian Public Health Association, Toronto, December, 1959.

THE ANTITUBERCULOUS ACTIVITY OF THIOAMIDE IN VITRO AND IN THE EXPERIMENTAL ANIMAL (MOUSE AND GUINEA PIG)[1]

Recent investigations by Rist and associates[2, 3] have demonstrated that the compound thioamide, alpha-ethyl-thioisonicotinamide[4] has activity against isoniazid-susceptible and -resistant tubercle bacilli in the test tube, as well as upon an infection induced in mice and guinea pigs with the H37Rv strain susceptible to isoniazid. Rist also reported that thioamide was ten times less active than isoniazid, but eight times more so than streptomycin in the experimental animal.

The present report describes an attempt to confirm the findings of the foregoing writers and also reports a study relative to the effect of thioamide and isoniazid[5] upon an infection in mice produced with thioamide-resistant H37Rv microorganisms. Furthermore, the *in vitro* activity of thioamide on an unclassified strain (Brownell) is discussed.

Effect of thioamide on M. tuberculosis and unclassified mycobacteria in vitro: Three types of liquid medium were used: Tween®-albumin, Proskauer and Beck's synthetic medium, and Proskauer and Beck's medium with 10 per cent horse serum added. The cultures used were the H37Rv strain and an unclassified strain (Brownell), which becomes pigmented when grown in the presence of light. The inoculum was 0.1 ml. of a fourteen-day culture, and growth recordings were made on the seventh, fourteenth, twenty-first, twenty-eighth, and thirty-fifth days. The results are recorded in table 1.

It may be noted in table 1 that on the thirty-fifth day the H37Rv strain, when grown in Tween-albumin medium, was partially resistant to 1.6 γ of thioamide per ml. and susceptible to 3.1 γ per ml. In Proskauer and Beck's medium the organisms were partially resistant to 0.05 γ per ml.; growth was questionable in 0.1 and 0.2 γ per ml., and the organisms were susceptible to 0.4 γ of thioamide per ml. The activity of the drug was diminished in Proskauer and Beck's medium plus 10 per cent horse serum, that is, the organisms grew in the presence of 6.2 γ of thioamide per ml. and were inhibited by 12.5 γ per ml. Tween-albumin medium also had a diminishing effect upon the activity of the drug. It is possible that the albumin in both the Proskauer and Beck medium plus serum and in the Tween-albumin medium may be the agent responsible for altering the inhibiting effect of the drug *in vitro*. The unclassified strain, Brownell, was resistant to 6.2 γ of thioamide per ml. and susceptible to 12.5 γ per ml. in Tween-albumin medium.

Emergence of thioamide-resistant tubercle bacilli: A strain of H37Rv tubercle bacilli was made resistant to thioamide by serial transfer in Tween-albumin medium containing increased concentrations of the drug. An inoculum of 0.1 ml. from the tube containing the highest concentration of thioamide, and showing good growth after fourteen days, was used for transfer.

After nine transfers there was growth in the 12.5 γ per ml. tube. From then on resistance increased more rapidly, and after the eighteenth transfer there was growth in the 200 γ per ml. tube.

Bactericidal action of thioamide on tubercle bacilli: The H37Rv strain of *M. tuberculosis* was seeded in Tween-albumin medium containing concentrations of 0.4, 0.8, 1.6, 3.1, 6.2, 12.5, and 25 γ of thioamide per ml. On the eighteenth day of incubation one double loopful of the culture from each tube was transferred onto the surface of non-drug–containing American Trudeau Society solid medium. On the thirty-fifth day growth recordings were made.

The following results were observed:

4+	growth from the 0.4 γ per ml. tube
4+	growth from the 0.8 γ per ml. tube
4+	growth from the 1.6 γ per ml. tube
4+	growth from the 3.1 γ per ml. tube
6	colonies from the 6.2 γ per ml. tube
1	colony from the 12.5 γ per ml. tube
0	growth from the 25.0 γ per ml. tube

From the foregoing it will be seen that, when the H37Rv strain of tubercle bacilli was incubated in the presence of thioamide in Tween-albumin medium for eighteen days, no growth was obtained from the tube containing 25.0 γ per ml., when subcultured on American Trudeau Society medium.

[1] This study was supported by research grants (E36 C12 and 13) from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, U. S. Public Health Service.

[2] Rist, N., *et al.:* Rev. tuberc., Paris, 1958, *22*, 278.

[3] Rist, N., *et al.:* Am. Rev. Tuberc., 1959, *79*, 1.

[4] Thioamide was received through the courtesy of Dr. Noël Rist, Institut Pasteur, Paris, France.

[5] The streptomycin and isoniazid were supplied by Chas. Pfizer & Company, Brooklyn, New York.

TABLE 1

IN VITRO EFFECT OF THIOAMIDE ON M. TUBERCULOSIS

Test Culture	Medium	Concentration	Growth in Days				
			7	14	21	28	35
		γ/ml.					
H37Rv, isoniazid-susceptible	Tween-albumin	0	3+	4+	4+	4+	4+
		0.1	3+	4+	4+	4+	4+
		0.2	3+	4+	4+	4+	4+
		0.4	±	+	3+	4+	4+
		0.8	±	±	±	3+	4+
		1.6	±	±	±	±	2+
		3.1	0	0	0	0	0
	Proskauer and Beck	0	3+	4+	4+	4+	4+
		0.0125	2+	3+	4+	4+	4+
		0.025	1+	2+	2+	3+	4+
		0.05	1+	1+	1+	2+	2+
		0.1	±	±	±	±	±
		0.2	0	±	±	±	±
		0.4	0	0	0	0	0
	Proskauer and Beck with 10% horse serum	0	2+	4+	4+	4+	4+
		0.1	2+	4+	4+	4+	4+
		0.2	2+	3+	4+	4+	4+
		0.4	2+	3+	4+	4+	4+
		0.8	1+	3+	4+	4+	4+
		1.6	0	2+	3+	3+	4+
		3.1	0	±	1+	2+	3+
		6.2	0	0	±	1+	1+
		12.5	0	0	0	0	±
Brownell (unclassified acid-fast)	Tween-albumin	0	3+	4+	4+	4+	4+
		0.1	3+	4+	4+	4+	4+
		0.2	3+	4+	4+	4+	4+
		0.4	3+	4+	4+	4+	4+
		0.8	3+	4+	4+	4+	4+
		1.6	2+	3+	4+	4+	4+
		3.1	1+	2+	3+	3+	4+
		6.2	±	2+	3+	3+	4+
		12.5	0	0	0	0	0

Effect of thioamide, isoniazid, and streptomycin on experimental tuberculosis in guinea pigs: Forty guinea pigs were infected subcutaneously with 0.2 ml. of a suspension containing 0.1 mg. dry weight of human H37Rv drug-susceptible tubercle bacilli. The animals were divided into four groups of 10 animals each, and therapy was begun immediately, using drug dosage as shown in table 2.

From the data in table 2 it may be seen that thioamide has limited activity against tuberculosis in the guinea pig and is less powerful than isoniazid or streptomycin when the average degree of tuberculosis is used for evaluating the effect of a drug.

Effect of isoniazid and thioamide on an infection in mice produced with H37Rv thioamide-resistant tubercle bacilli: Thirty CF_1 white female mice were infected intravenously with 0.1 ml. (0.3 mg. dry weight) of H37Rv tubercle bacilli made resistant to thioamide. Ten mice remained untreated. Each of 10 mice received 0.025 mg. of isoniazid daily mixed with powdered mouse pellets, and each of 10 received 0.25 mg. daily of thioamide mixed with their food. Treatment was started immediately after infection and continued for forty days. All survivors were killed on the fortieth day. The results of the experiment are recorded in table 3.

It will be noted in table 3 that the thioamide had no effect upon disease produced by tubercle bacilli resistant to thioamide, when compared with the control animals, whereas isoniazid had excellent effect. The control animals and those

TABLE 2

AVERAGE DEGREE OF TUBERCULOSIS IN GUINEA PIGS TREATED WITH TH 1314, ISONIAZID, AND STREPTOMYCIN

Drugs and Dosage	Number of Animals	Average Index of Gross Tuberculosis*				
		Lungs	Liver	Spleen	Nodes	Total
Controls	10	2.85	2.85	2.55	3.45	11.7
TH 1314 10 mg./kg.	10	1.45	0.85	0.85	2.5	5.65
Streptomycin 20 mg./kg.	10	0.1	0	0	1.3	1.4
Isoniazid 2.5 mg./kg.	10	0	0	0	0	0

* Based on value of 0 to 4+ for each of lungs, liver, spleen, and lymph nodes according to amount of gross tuberculous disease. Maximum was 16 for each animal.

H37Rv drug-susceptible strain of tubercle bacilli *in vitro* in a concentration of 3.1 γ per ml. in Tween-albumin medium, and of 0.4 γ per ml. in Proskauer and Beck's medium. In the latter with 10 per cent horse serum added, 12.5 γ per ml. were required. The Brownell strain of unclassified mycobacteria was inhibited by 12.5 γ per ml. of the drug in Tween-albumin medium.

The H37Rv strain was made resistant to 200 γ of thioamide per ml. after eighteen transfers in drug-containing Tween-albumin medium.

The drug showed definite bactericidal activity on H37Rv tubercle bacilli in Tween-albumin medium containing 6.2 and 12.5 γ per ml.

The thioamide had limited activity in guinea pigs infected subcutaneously with H37Rv tubercle bacilli and treated with 10 mg. per kg. per day. The drug was much less active than isoniazid and streptomycin.

The thioamide had no effect upon an infection induced in mice with thioamide-resistant H37Rv tubercle bacilli, whereas isoniazid had excellent activity in these circumstances.

TABLE 3

ISONIAZID AND TH 1314 IN MICE INFECTED WITH TH 1314-RESISTANT TUBERCLE BACILLI

Infecting Strain	Treatment	Survival Days After Infection	Mortality at Days		Average Gross Lung Disease*
			30	40	
			Per Cent	*Per Cent*	
H37Rv, resistant to TH 1314	Controls	19, 20, 22, 23, 28, 28, 29, 29, 30, 30	100	—	3.9
	Isoniazid 0.025 mg.	(All killed at 40 days)	0	—	0.7
	TH 1314 0.25 mg.	20, 20, 20, 22, 22, 25, 26, 27, 27, 29	100	—	3.7

* Based on a score of 0 to 4+.

treated with thioamide had a 100 per cent mortality at thirty days, with an average degree of tuberculosis in their lungs of 3.9 and 3.7, respectively. Those treated with isoniazid survived and were killed on the fortieth day, showing an average of 0.7 disease in their lungs.

In summary: The drug alpha-ethyl-thioisonicotinamide (thioamide) inhibited the growth of the

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ANTITUBERCULOUS ACTIVITY OF RIMINO COMPOUND OF THE PHENAZINE SERIES[1]

This report is concerned with observations of the antituberculous activity of rimino compound (B663 of the phenazine series) *in vitro* and *in vivo*.[2, 3, 4] In the studies, the effect of rimino compound on an intracardially induced infection in guinea pigs with H37Rv isoniazid-susceptible, catalase-positive tubercle bacilli was evaluated. Studies were also made with the bovine strain 4228-4 (which was isoniazid susceptible, catalase positive) and its counterpart, an isoniazid-resistant, catalase-negative strain 4228-4-435.

Effect of rimino compound on M. tuberculosis in vitro: Three types of liquid medium were used: Tween®-albumin, Proskauer and Beck synthetic medium, and Proskauer and Beck medium with 10 per cent horse serum. The H37Rv isoniazid-susceptible and -resistant strains and an unclassified strain that is pigmented when grown in the presence of light (Brownell) were the test cultures. The inoculum was 0.1 ml. of a fourteen-day culture grown on Tween-albumin medium. Growth recordings were made on the seventh, fourteenth, twenty-first, twenty-eighth, and thirty-fifth days. The results are recorded in table 1.

It will be noted in table 1 that on the thirty-fifth day of growth the H37Rv isoniazid-susceptible strain, when cultured in Tween-albumin medium, grew in the presence of 0.16 γ of rimino compound per ml. and was inhibited by 0.31 γ per ml. In Proskauer and Beck medium the organisms grew in 0.16 γ of rimino compound per ml. and were inhibited by 0.31 γ per ml. In Proskauer and Beck medium plus 10 per cent horse serum, there was growth in 1.25 γ of rimino compound per ml. and questionable growth in the tube containing 2.5 γ per ml. The H37Rv strain resistant to isoniazid, when grown in Tween-albumin medium, was resistant to 0.16 γ of rimino compound per ml. and susceptible to 0.31 γ per ml. The unclassified strain (Brownell), when grown in Tween-albumin medium, was resistant to 0.62 γ of rimino compound per ml. and susceptible to 1.25 γ per ml.

Emergence of drug-resistant tubercle bacilli: A strain of H37Rv tubercle bacilli was cultured in Tween-albumin medium, and serial transfers were made in medium containing increased concentrations of the drug. An inoculum of 0.1 ml. from the tube containing the highest concentration of B663 and showing good growth after fourteen days was used for transfer.

After fourteen transfers there was no loss of susceptibility to rimino compound. Growth appeared in the 0.31 γ per ml. tube and there was no growth in the 0.62 γ per ml. tube.

Bactericidal action of rimino compound on tubercle bacilli: The H37Rv strain of *M. tuberculosis* was seeded in Tween albumin medium containing the following concentrations of B663: 0.01, 0.02, 0.04, 0.08, 0.16, 0.31, 0.62 γ per ml. On the fourteenth day of incubation one double loopful of the culture from each tube was seeded on the surface of non-drug–containing American Trudeau Society solid medium.

On the twenty-eighth day growth recordings were made and the following results observed:

4+ growth from the 0.02 γ per ml. tube.
4+ growth from the 0.04 γ per ml. tube.
2+ growth from the 0.08 γ per ml. tube.
7 colonies from the 0.16 γ per ml. tube.
11 colonies from the 0.31 γ per ml. tube.
No growth from the 0.62 γ per ml. tube.

From the data it may be seen that, when cultured in the presence of 0.62 γ of rimino compound per ml., the H37Rv strain had no viable cells when a double loopful of the culture was streaked on the surface of American Trudeau Society solid medium.

Effect of rimino compound on experimental tuberculosis in guinea pigs infected with isoniazid-sus-susceptible and -resistant tubercle bacilli: One hundred and twenty-five female guinea pigs weighing approximately 500 gm. each were infected intracardially with 0.2 ml. of a suspension containing 0.1 mg. (dry weight) of tubercle bacilli. Twenty-five animals were infected with H37Rv isoniazid-susceptible, 50 with bovine 4228-4 isoniazid-susceptible, and 50 with bovine 4228-4-435 isoniazid-resistant tubercle bacilli. These infected animals were subdivided: 5 animals for each of the control groups and 10 animals for each of the treated groups. The groups were as follows:

Group 1—Controls. H37Rv, isoniazid-susceptible
Group 2—5 mg. per kg. per day of rimino compound

[1] This study was supported by research grant E36 C13 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, U. S. Public Health Service.

[2] The rimino compound (B663) was kindly supplied through Dr. Vincent C. Barry by J. R. Geigy, S. A. Basle, Switzerland.

[3] BARRY, V. C., *et al.*: J. Pharm. & Pharmacol., 1956, *8*, 1089.

[4] BARRY, V. C., *et al.*: Nature, London, 1957, *179*, 1013.

TABLE 1

IN VITRO EFFECT OF RIMINO COMPOUND

Test Culture	Medium	Concentration	Growth in Days				
			7	14	21	28	35
		γ/ml.					
H37Rv isoniazid-susceptible	Tween-albumin	0	2+	4+	4+	4+	4+
		0.01	2+	3+	4+	4+	4+
		0.02	2+	3+	3+	4+	4+
		0.04	1+	2+	3+	4+	4+
		0.08	1+	2+	2+	4+	4+
		0.16	±	1+	1+	3+	3+
		0.31	0	0	0	0	0
	Proskauer and Beck	0	2+	4+	4+	4+	4+
		0.01	2+	4+	4+	4+	4+
		0.02	2+	4+	4+	4+	4+
		0.04	2+	3+	4+	4+	4+
		0.08	2+	3+	4+	4+	4+
		0.16	±	±	2+	3+	3+
		0.31	0	0	0	0	0
	Proskauer and Beck with 10% horse serum	0	3+	4+	4+	4+	4+
		0.01	3+	4+	4+	4+	4+
		0.02	2+	3+	4+	4+	4+
		0.04	2+	3+	4+	4+	4+
		0.08	2+	3+	4+	4+	4+
		0.16	1+	3+	4+	4+	4+
		0.31	1+	3+	4+	4+	4+
		0.62	1+	2+	3+	4+	4+
		1.25	±	1+	2+	3+	4+
		2.5	±	±	±	±	±
H37Rv isoniazid-resistant	Tween-albumin	0	2+	4+	4+	4+	4+
		0.01	1+	3+	4+	4+	4+
		0.02	1+	3+	4+	4+	4+
		0.04	±	2+	3+	4+	4+
		0.08	±	1+	2+	3+	4+
		0.16	0	±	1+	2+	2+
		0.31	0	0	0	0	0
Brownell unclassified Mycobacterium	Tween-albumin	0	2+	4+	4+	4+	4+
		0.01	1+	4+	4+	4+	4+
		0.02	1+	2+	3+	3+	4+
		0.04	±	1+	2+	2+	3+
		0.08	±	1+	1+	1+	2+
		0.16	0	±	±	1+	1+
		0.31	0	±	±	1+	1+
		0.62	0	±	±	±	1+
		1.25	0	0	0	0	0

Group 3—10 mg. per kg. per day of rimino compound
Group 4—Controls. Bovine 4228-4, isoniazid-susceptible
Group 5—5 mg. per kg. per day of rimino compound
Group 6—10 mg. per kg. per day of rimino compound
Group 7—Controls. Bovine 4228-4, isoniazid-susceptible
Group 8—5 mg. per kg. per day of isoniazid
Group 9—10 mg. per kg. per day of isoniazid
Group 10—Controls. Bovine 4228-4-435, isoniazid-resistant
Group 11—5 mg. per kg. per day of rimino compound
Group 12—10 mg. per kg. per day of rimino compound
Group 13—Controls. Bovine 4228-4-435, isoniazid-resistant

TABLE 2

EFFECT OF RIMINO COMPOUND AND ISONIAZID ON GUINEA PIGS INFECTED INTRACARDIALLY WITH ISONIAZID-SUSCEPTIBLE AND -RESISTANT TUBERCLE BACILLI

Group	Infecting Organism and Susceptibility to Isoniazid		Number of Animals	Drug and Dosage*		Average Days of Life
	Susceptible	Resistant		Rimino Compound	Isoniazid	
1	H37Rv		5	Controls		36
2	H37Rv		10	5.0 mg.		28
3	H37Rv		10	10.0 mg.		34
4	Bovine 4228-4		5	Controls		27
5	Bovine 4228-4		10	5.0 mg.		42
6	Bovine 4228-4		10	10.0 mg.		47
7	Bovine 4228-4		5		Controls	27
8	Bovine 4228-4		10		5.0 mg.	575 Still living
9	Bovine 4228-4		10		10.0 mg.	575 Still living
10		Bovine 4228-4-435	5	Controls		28
11		Bovine 4228-4-435	10	5.0 mg.		24
12		Bovine 4228-4-435	10	10.0 mg.		32
13		Bovine 4228-4-435	5		Controls	28
14		Bovine 4228-4-435	10		5.0 mg.	26
15		Bovine 4228-4-435	10		10.0 mg.	28

* Dosage mg. per kg. per day of isoniazid and rimino compound.

Group 14—5 mg. per kg. per day of isoniazid
Group 15—10 mg. per kg. per day of isoniazid

Therapy was begun immediately after infection. Each animal received the prescribed amount of drug in small gelatin capsules once a day by mouth by forced feeding. Treatment was continued until the animals died, and the survival time in terms of average days of life was computed and recorded as presented in table 2. The 10 animals in each of *Groups 8* and *9* that received isoniazid were allowed to survive in order to note the rate of relapse from their infection after cessation of two years of continuous isoniazid therapy, and will constitute part of a study to be reported at a later date.

It will be noted in table 2 that there was little, if any, difference in the survival time of four of the groups of animals treated with rimino compound (*Groups 2, 3, 11, 12*) and those that received no treatment (*Groups 1, 4, 10*). Likewise, in those animals (*Groups 14, 15*) infected with isoniazid-resistant organisms and treated with isoniazid, there was no difference in survival time when compared with the nontreated control animals (*Group 13*). *Groups 5* and *6*, infected with bovine-susceptible strain 4228-4, showed approximately a twofold superior survival time when compared with the control animals (*Group 4*). The animals in each of *Groups 8* and *9*, infected with bovine isoniazid-susceptible tubercle bacilli and treated with 5.0 and 10.0 mg., respectively, of isoniazid once a day, have shown a dramatic survival time of 575 days as compared with the controls (*Group 7*) which survived 27 days.

The beneficial effect of rimino compound on

tuberculosis in guinea pigs, as noted by Barry and associates,[4] in contrast to the findings reported in this paper, possibly may be explained in part by the different methods used in infecting the animals. Barry infected his guinea pigs subcutaneously, whereas the animals in this study were infected intracardially. With the latter model a more widely disseminated virulent infection is produced which is quite refractory to drug therapy unless the drug used for treatment has antituberculous effectiveness of the order of isoniazid or streptomycin. Practically all of the animals that died of their disease while on rimino compound therapy had symptoms of brain damage from the infection, manifested by paralysis of the hind legs and rotation of the head. This is in marked contrast to the excellent results obtained with isoniazid in animals infected by the same route with isoniazid-susceptible organisms. Like Barry, the present writers noted that the subcutaneous fat became a deep yellow in some of the animals while they were receiving therapy with the rimino compound.

In summary: Rimino compound (B663) of the phenazine series inhibits the growth *in vitro* of, and has definite bactericidal activity for, H37Rv tubercle bacilli. The H37Rv strain loses its susceptibility to this compound when grown in medium containing subinhibitory concentrations. The drug had little effect in the dosage used upon an intracardially induced tuberculous infection in guinea pigs. On a weight-for-weight basis, isoniazid was superior to rimino compound in the treatment of intracardially infected guinea pigs.

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Books

LE PNEUMOTHORAX SPONTANE NON TUBERCULEUX DE L'ADULTE ET SON TRAITEMENT. By André Meyer, J. P. Nico, and J. Carraud. Pp. 144, Masson et Cie., Paris, 1958. Price, 1950 fr.

This book provides a fairly complete coverage on the subject of nontuberculous spontaneous pneumothorax. It begins with a chapter on history which traces the development of our present concepts on the pathophysiology and treatment of this condition. This is followed by six chapters discussing, in order, the etiology, symptomatology course, complications, clinical forms, and differential diagnosis. The last and longest chapter is devoted to treatment.

In their introduction to the book, the authors state that they have observed 150 cases of spontaneous pneumothorax in recent years. Sixty-five cases were studied intensively and, of these, a group of 53 cases which were admitted to the Respiratory Service of the Boucicaut Hospital between 1949 and 1954 forms the basis of the book. During the same six-year period, they encountered only 4 cases of spontaneous pneumothorax of tuberculous origin. The infrequency of tuberculosis in the pathogenesis of spontaneous pneumothorax confirms other recent reports. The authors' experience is also similar to that of other workers in this field in that nontuberculous spontaneous pneumothorax is a disease mainly of young adults, males predominating 10 to one over females, and the right side involved slightly more often than the left. Though occasionally asymptomatic, pain and dyspnea are its outstanding symptoms. From a review of the literature, the authors make the interesting observation that spontaneous pneumothorax occurring during a therapeutic pneumoperitoneum is always on the right side.

In general, the discussion on etiology is very good. But, while such rare and remote factors as emotions and electric shock are brought forth, no mention is made of the theory that ischemia of the lung periphery may have a role in the genesis of blebs or bullae that rupture to cause pneumothorax. As is to be expected, the course of these 53 patients depended on the underlying pathology. It was usually benign when there was a localized lesion, and complications were more frequent when the pulmonary parenchyma had some generalized disease or loss of elasticity, or when the causative bulla was very large and had a greatly thickened wall. The chief complications were respiratory insufficiency, tension pneumothorax, hemopneumothorax, sterile or infected pleural effusion, and cardiac failure. The authors point out that chronicity—which they define as a pneumothorax not resorbed within two to three months—and relapses are not uncommon when the underlying lung is emphysematous or silicotic, whereas in the asthmatic patient (and here reference is made to the work of Turiaf) relapses of the pneumothorax are frequent but chronicity is rare. Incidentally, patients with spontaneous pneumothorax overlying generalized emphysema are usually more than forty years old. Those with silicosis usually have only a localized or partial pneumothorax due to the many pleural adhesions.

The treatment recommended for nontuberculous spontaneous pneumothorax consists essentially of bed rest supplemented after the fourth day, if necessary, by daily needle aspirations until the pulmonary re-expansion is complete. If by the third week expansion is not achieved, a pleuroscopy (thoracoscopy) is performed, and a chest catheter is passed through the pleuroscope and connected to a water seal with or without continuous or intermittent suction. Talc poudrage, usually through the pleuroscope, is their treatment of choice for cases requiring pleural symphisis—recurrent cases or cases with diffuse blebs or bullae. For gross localized lesions or recurrences therefrom, conservative resection is advised. Although a 25–30 per cent pneumothorax could very well be treated with bed rest alone, for the more severe cases of pulmonary collapse this reviewer favors a more aggressive approach with a brief trial of needle aspirations which, if unsuccessful in bringing out the collapsed lung in twenty-four hours, should be followed by early institution of an intercostal chest catheter draining to a water seal and, preferably, connected to suction of 10 to 20 cm. of water. Such a regimen will reduce the time of treatment and the hospital morbidity from weeks to days.

Pleuroscopy finds more favor with the authors than it does with most chest surgeons in this country. The author's viewpoint seems fully justified considering that, of 15 cases pleuroscoped with negative roentgenographic findings, they found blebs or bullae in 11 cases, other pathology in 3 cases, and only one case was completely normal.

The gravity of hemopneumothorax is rightly emphasized, and the bleeding is thought to origi-

nate from vascular adhesions which are torn by the pneumothorax. The treatment is well outlined, but the fibrinolytic enzymes receive no more than one line.

With the division of the subject matter into eight separate chapters, a fair amount of repetition becomes inevitable. Although this may seem objectionable to an efficiency expert, I do not view it with criticism, especially since throughout the book the sequence of presentation remains smooth and the clarity of expression is uninterrupted. The authors subscribe to the "equal time" policy in presenting points of view at variance with their own and, indeed, their excellent bibliography of 282 references speaks for itself.

The printing is of high quality, and the 32 illustrations, most of which are roentgenograms, are quite good. The book lacks an index. The binding is below American standards—the reviewer's is already suffering from progeria. A paper knife should accompany the book to help you open the edges of the pages.

In summary, despite some minor shortcomings, chest physicians and surgeons as well as others interested in spontaneous pneumothorax will find this work one of the best available references on the subject.

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An Experimental Investigation of Methods for the Cultivation of *Mycobacterium Tuberculosis* from Sputum. By Rolf Saxholm. Pp. 148, Oslo University Press, Oslo, 1958.

This book, based on Dr. Saxholm's experiments comparing various concentration methods for the cultivation of tubercle bacilli from sputum, also includes a brief historical résumé.

After describing the generally used concentration techniques for tubercle bacilli and their merits and differences, the author discusses the properties of mucolytic enzymes, the quaternary ammonium compounds, and defines the differences of the germicidal activity of these agents.

Dr. Saxholm provides detailed information about the materials and methods used and the results obtained. The majority of the experiments were carried out with quaternary ammonium compounds. In search for an optimal method, a number of variables such as different culture media and several pH ranges were studied, and different batches of enzymes and quats were compared. Information about the influence of storage on the media and specimens, and the longevity of tubercle bacilli in sputum is also given. The methods for the statistical evaluation of the experiments are explained.

The extensive discussion is followed by the summary. The references to the literature are complete and are followed by fifty-eight informative tables.

Dr. Saxholm's extensive and explicit studies will be of considerable value to any investigator concerned with the cultivation of tubercle bacilli from sputum.

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Physiotherapy in Chest Diseases. By Torsten Bruce, Caroline Reutersward, and Birgit Westin. Pp. 98, National Association for the Prevention of Tuberculosis, London, 1958. Price, 12 s. 6 d.

This manual describes how the Söderby Hospital in Uttran, Sweden, has adopted the British pioneering method of physiotherapy in chest diseases. The principles are described for use in patients with tuberculosis, but it is emphasized that they are just as useful for patients with any sort of pulmonary surgery and also in the operative treatment of heart disease.

The first chapter by Dr. Bruce gives a very clear medical orientation for the guidance of physiotherapists. He points out that the four goals of physiotherapy are: to facilitate breathing, to promote expectoration, to prevent adhesions, and to improve bronchial drainage. For these, he uses the rather unforgettable term "bronchial toilet." In addition, physiotherapy is aimed at prevention of limitation of motion in the arm and shoulder.

The physiotherapy techniques are extremely practical. Methods of postural drainage are described here which are effective yet comfortable enough so that patients of any age may use them. It was once thought that postural drainage necessitated up-ending the patient, but methods are described in this book whereby the patient lies comfortably in bed. The importance of close cooperation between the physiotherapist and the ward nurse is emphasized and, indeed, this is the secret of continuity of care in these patients.

The book would be more usable if the exercises were all tabulated on one or two pages rather than spread throughout the book and mixed in with the text. However, since the book is only 98 pages, this is not too serious a criticism. From pages 85

to 98, there are excellent reproductions of roentgenograms illustrating points in the text.

This book makes a strong case for the use of physiotherapy in chest diseases and, certainly, results have proved physiotherapy to be of importance. However, in this country these valuable techniques are often overlooked. The reviewers hope this book has a wide distribution because lack of use of these exercises amounts to serious neglect of certain patients.

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BOOKS RECEIVED

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American Trudeau Society

Medical Section of the National Tuberculosis Association

NOTICES

The following dates and locations of the next five annual meetings of the National Tuberculosis Association and the American Trudeau Society have been selected and approved:

1960—Los Angeles, California, May 15–20
1961—Cincinnati, Ohio—May 21–26
1962—Miami Beach, Florida—May 20–25
1963—Denver, Colorado—May 12–17
1964—New York, New York—May 24–29

NURSES' PARTICIPATION IN TUBERCULIN AND OTHER SKIN-TESTING PROGRAMS

There is evidence that many more nurses will be involved in tuberculin-testing programs in the future than at present. This is the consensus of the National League for Nursing–National Tuberculosis Association Committee on Tuberculosis Nursing which met at NLN Headquarters on November 13, 1959.

The committee's pronouncement, made before the Arden House Conference on Tuberculosis held in Harriman, New York, November 29–December 2, 1959, sponsored by the NTA and the Public Health Service, makes it crystal clear that nurses in increasing numbers will be called upon to administer tuberculin tests.

The report of the conference and the recommendations were published in the February issues of the NTA *Bulletin* and *Public Health Reports*. They are directed toward one goal, *the elimination of tuberculosis in the U.S.A.* The major recommendation "is a program for widespread application of chemotherapy as a public health measure. . . ."

This recommendation obviously implies an adequate case-detection program which includes the identification of the *infected population*; and the tuberculin test is the only means known at this time for doing this. Therefore, nurses in public health agencies, hospitals, industries, schools, physicians' offices, and others will frequently have to be depended upon to administer and read the test.

Before nurses are assigned to tuberculin-testing programs, it will be necessary in many instances to clear with state and local medical societies and medical advisory committees of visiting nurse associations. A look at nurse practice acts in relation to the medical practice acts is indicated. Some nurse practice acts are restrictive but, frequently, legal interpretation brings them in line with prevailing practice in a given community. For example, it may be unlawful for nurses to "puncture" a patient's skin. How nurses have practiced all of these years without doing this is hard to say. State nurses' associations may be helpful in pursuing the problem, as many have been active in this area. The agency responsible for the program must be able to assure the nurses participating in it that they are not violating the nurse practice act and that the nurses are fully covered by professional liability insurance for this type of procedure.

Questions have been raised regarding the reading of the test by nurses. The nurse doing the reading is not making a diagnosis. She observes and records the size of the area of induration or its absence. On the basis of this information the physician will decide whether the test is positive or negative. Authorities will vary the point at which they set limits. Some will call tuberculin tests positive if the induration is 6 mm. or more in diameter, others if it is 8 or 10 mm. or more. Local conditions and the kind of information being sought will determine what limit is to be used. The larger the induration the greater the need for immediate follow-up examination of the individual and his associates.

No tuberculin-testing activity is successful unless the reactors are further examined for the presence of active tuberculosis, and their associates studied for possible source cases.

Nurses need to learn how to give the test, to practice intradermal administration in order to gain skill, and to read the results of the test with accuracy. Your state or local tuberculosis association or your state or local health department may be able to give you the help you need. Interorganization committees on tuberculosis nursing of state and local leagues for nursing and tuberculosis associations may wish to plan practical demonstrations or otherwise assist in preparing nurses to give this service.

Nurses who recognize the importance of their participation in tuberculin-testing programs in helping to study the epidemiology of tuberculosis will find this activity exciting and satisfying.

APPOINTMENT OF MEDICAL DIRECTOR OF AMERICAN TRUDEAU SOCIETY

Dr. James W. Raleigh of the pulmonary diseases service of the Veterans Administration, Washington, D. C., has been appointed as medical director of the American Trudeau Society, medical section of the National Tuberculosis Association. Dr. Raleigh will assume his post on June 15.

The position is a new one, created to coordinate the activities of the growing medical section of the National Tuberculosis Association, which now has a membership of 5,200 physicians and other scientists in North America, South America, Europe, and Asia.

A native of Brooklyn, Dr. Raleigh attended Regis High School in New York City, and was graduated in 1933 from Holy Cross College, Worcester, Massachusetts. He received his medical degree from Long Island College of Medicine, Brooklyn, in 1939, and interned in St. Mary's Hospital there. Later, he was on the staff of Stony Wold Sanatorium, Lake Kushaqua, New York.

In 1947 Dr. Raleigh went to the Veterans Administration Hospital, Sunmount, New York, as assistant chief of the tuberculosis service and became chief of the pulmonary disease service in 1953. He left Sunmount in 1958 to join the Central Office of the Veterans Administration as head of clinical research in pulmonary diseases and as executive secretary of the Veterans Administration–Armed Forces Cooperative Study on the Chemotherapy of Tuberculosis.

Dr. Raleigh is a Diplomate of the American Board of Internal Medicine. While in Washington he was assistant clinical professor of medicine at Georgetown University Medical School. He was previously assistant clinical professor of medicine at the University of Vermont College of Medicine.

ANNUAL SYMPOSIUM OF PULMONARY DISEASES AT FITZSIMONS GENERAL HOSPITAL

The Thirteenth Annual Symposium on Pulmonary Diseases will be held September 19–23, 1960, under the co-sponsorship of Fitzsimons General Hospital, the University of Colorado School of Medicine, and the American Trudeau Society. The tuition for the course is $5.00. Further information may be obtained from the University of Colorado or from Col. James A. Wier, Chief, Pulmonary Disease Service, Fitzsimons Army Hospital, Denver 30, Colorado.

THE AMERICAN REVIEW
OF RESPIRATORY DISEASES

Clinical and Laboratory Studies of Tuberculosis and Respiratory Diseases

ABSTRACTS

Edited by EDWIN D. KILBOURNE

VOLUME 81 | May 1960 | ABST. No. 5

The Editor recognizes the inevitable incompleteness and arbitrary selectivity of any abstracting service, including this one. It is his belief that the usefulness of the present Abstracts section will be enhanced if the reader is informed of the general purpose of the abstracts and the criteria employed in their selection.

It is the purpose of the Abstracts section to acquaint the diversified and international readership of the REVIEW with the gist of original and scientific articles which are related to the general subjects of tuberculosis or respiratory diseases implicit in the title of the journal. In this connection, 40 abstracters systematically review 207 journals which represent 31 countries and which are published in 18 languages. In most instances, abstracters cover journals in fields of their special competence. It is evident that the diverse interests of the readers of the REVIEW dictate a balanced selection of clinical, laboratory, and epidemiologic studies. Such selection will often preclude the publication of articles equal in scientific merit to many presented here.

In the publication of abstracts, cognizance is taken of the extent to which the journals yielding them are circulated. Articles from journals of wide circulation may be mentioned by title only. This space-saving device is also employed for articles which defy condensation without the omission of essential data or discussion. The attention of readers is thus directed to the existence of papers which are no less important because they are not summarized.

(Requests for further information on abstracted articles should be directed to the author of the article at the address indicated by an asterisk (*) in each abstract title.)

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NONTUBERCULOUS THORACO-PULMONARY DISEASE

Evaluation of the Results of Specific Hyposensitisation Therapy in Cases of Respiratory Allergies Treated in Sawai Man Singh Hospital, Jaipur. R. M. KASLIWAL*, K. D. GUPTA, L. M. SANGHVI, and I. C. SOGANI. *Indian J. Chest Dis.*, July, 1959, 1: 17–23. (*Sawai Man Med. College, Jaipur, India)

Results of treatment with specific hyposensitization have been reported in 106 cases of respiratory allergies. Specific therapy was given by injecting the causal allergen subcutaneously in graduated doses. Evaluation has been made at four levels of treatment: at the end of 3 months, 6 months, 1 year, and 1½ years. It was seen that cases of bronchial asthma responded best and, of these, 48 per cent showed complete improvement, and 48 per cent partial improvement; 4 per cent proved failures. Allergic rhinitis was most difficult to treat and, of these, 13 per cent did not improve at all. The number of hay-fever patients was too small for statistical evaluation.

It has been concluded that: (*1*) the longer the duration of treatment, the greater the number of patients who benefit; (*2*) most patients who are likely to benefit show signs of improvement in the first 6 months of therapy; and (*3*) any patient who does not show any relief after the first few months of therapy either will not respond to therapy or requires a revaluation of his case.

Familial Arteriovenous Aneurysms of the Lung (in German). CHR. HEDINGER*. *Schweiz. med. Wchnschr.*, August 8, 1959, 89: 846–851. (*Kantonsspital, Winterthur, Switzerland)

Six cases are reported of arteriovenous aneurysms of the lung associated with hereditary hemorrhagic teleangiectasis (Osler). Four of the patients belonged to the same family.

J. HAAPANEN

Traumatic Rupture of the Aorta (in Norwegian). A. FLATMARK*. *Nord. med.*, August 6, 1959, 62: 1195. (*Tønsberg, Norway)

A 10-year-old boy sustained a nonpenetrating thoracic injury by falling on ice. He was admitted to the hospital with severe pain in the chest and cyanosis, but without signs of shock. The roentgenogram of the chest was normal. The pain was relieved by left-sided paravertebral procaine block. He was quite well until 7½ hours after the injury, when he was seized by a sudden severe chest pain, collapsed, and died. Autopsy revealed a rupture at the base of the aorta, with a dissecting aneurysm emerging to the diaphragm, and the right pleural cavity was full of blood.

J. HAAPANEN

Pleurisy with Effusion in Rheumatoid Arthritis. P. R. LEE*, H. S. SOX, F. S. NORTH, and G. A. WOOD. *A. M. A. Arch. Int. Med.*, October, 1959, 104: 634–639. (*Palo Alto Med. Clinic, Calif.)

Pleural effusion occurring concurrently with rheumatoid arthritis may be a systemic manifestation of the latter disease. Although other causes (especially neoplasm, tuberculosis, systemic lupus erythematosus, and rheumatic fever) should be sought, the effusion can be explained on the basis of rheumatoid arthritis alone. Two patients developed large pleural effusions as systemic manifestations of their rheumatoid arthritis. Outstanding features of the 2 cases included the presence of subcutaneous nodules, a high titer of rheumatoid factor in the serum, long-standing disease, joint destruction on roentgenogram, patient age more than 55 years, and persistence of pleural effusion. In both patients, clinical and roentgenographic improvement occurred coincident with chloroquine therapy.

W. J. STEININGER

Urinary 17-Ketosteroids in Indian Asthma Patients. D. N. SHIVPURI*, T. A. VENKITASUBRAMANIAN, and R. VISWANATHAN. *Indian J. Chest Dis.*, July, 1959, 1: 42–49. (*V. P. Chest Institute, U. of Delhi, India)

The daily output of urinary 17-ketosteroids was estimated in 68 patients who attended the Asthma Clinic of the Patel Chest Institute; 37 were males, and 31 females; ages ranged from 5 to 65 years. The ranges and the mean values were found to be much lower in asthma patients than the average values for normal subjects. There were no significant differences in the values between the different types of asthma or the different age groups above the age of 13.

A definite increase was seen during winter in the daily output of urinary 17-ketosteroids in a few asthma patients in whom the estimations were done during winter as well as summer. There was no change in the values after a subcutaneous injection of 0.5 ml. of epinephrine (Adrenalin®) given during an induced attack or during the remission period. There was a slight but definite increase in these values after administration of 25 mg. of corticotropin during induced attacks or during remission in the same patients. There was also slight but constant increase in the creatinine/uric-acid ratio in these patients on the days they received hormones.

Atropine Cigarettes in Asthma and Emphysema. H. HERXHEIMER*. *Brit. M. J.*, August 15, 1959, No. 5145: 167–171. (*Free U. of Berlin, Germany)

Twenty-three patients with asthma or emphysema were tested with cigarettes containing 1.45

mg. of atropine, and 16 were given 0.5-mg. atropine cigarettes in 48 experiments. Ten patients were tested with both strengths of cigarettes. In the great majority, the vital capacity increased after smoking, often by a substantial amount. The increase varied between 5 and 48 per cent and, in 52 of the 62 experiments, increased by 10 per cent or more. Blank cigarettes did not change the vital capacity by more than 3 per cent. The increase in the vital capacity was maintained for up to 3 hours. The results with cigarettes containing stramonium were similar to those with atropine cigarettes. A similar result was noted with wet atropine aerosols, provided the droplets were small enough to penetrate into the finer bronchioli.

E. A. RILEY

Fibrinous Bronchitis: Its Mechanism, Treatment, and Implications. V. TAPPAN*. *A. M. A. J. Dis. Child.*, October, 1959, 98: 514. (*Yale U. School of Med., New Haven, Conn.)

During the past 2 years at the Yale Medical Center, 2 patients aged 13 and 15 years, respectively, with pulmonary stenosis and interauricular septal defect, developed fibrinous casts following cardiac surgery. It is suggested that fibrin cast formation is to be traced: (*1*) to the pathologic transudation of plasma fluids and plasma proteins into the peripheral respiratory tract, and (*2*) to the polymerization of activated fibrinogen under the special conditions created by local respiratory acidosis, and by disturbed intrapulmonary pressures. The mechanism is believed to be one of acute overloading with blood of anatomically inadequate pulmonary circulation in individuals with limited gas exchange who are breathing against acutely imposed obstructions.

Based upon this pathogenic concept, the older boy has been treated for more than 2 years with beneficial effects: (*1*) by the use of continuous broncholysis, and (*2*) by alkalization in amounts designed to keep him in a state of mild alkalosis. This concept and therapeutic approach are thought to have useful implications in the general area of pathologic conditions of the lungs.

M. J. SMALL

Chronic Cor Pulmonale in Pulmonary Tuberculosis (in Spanish). I. AELLO PARODI* and R. PEREZ TAMAYO. *Rev. mex. Tuberc.*, November–December, 1958, 19: 516–533. (*National School of Medicine, Mexico)

Among 1,632 autopsies performed from 1954 to 1957, 214 cases of pulmonary tuberculosis were studied, with special reference to the degree of obstruction to the pulmonary circulation and the production of cor pulmonale. Cases with minimal or very localized lesions producing no obstruction were eliminated, as were also those with left-side lesions. Right ventricular hypertrophy was diagnosed when the average thickness of this wall was 5 mm. or more. In addition, the diagnosis of right ventricular dilatation was made when a minimal difference of 3 cm. or more between the inflow and outflow tracts of this chamber was found. The diagnosis of mixed hypertrophy and dilatation was made when both of these criteria appeared simultaneously in the same heart. The diagnosis of cardiac insufficiency was made only in those cases presenting chronic passive congestion of the liver, ascites, and edema, and clinical signs of congestive heart failure. Cases were classified as treated and untreated. Treated cases were those which received antituberculosis chemotherapy for a period of at least 3 months.

Of the 214 cases studied, 60 (28 per cent) showed some of the above specified alterations of the right ventricle, distributed as follows: ventricular dilatation, 28 cases (13.0 per cent); pure ventricular hypertrophy, 14 (6.5 per cent); combined dilatation and hypertrophy, 14 (6.5 per cent). These were classified as cor pulmonale. Of these 60 cases, 44 were males, with an average age of 38.2 years at the time of death, and 16 females, with an average age of 29 years at the time of death. In the 60 cases, the average course was 2 years and 4 months.

Of the 154 cases not presenting cor pulmonale, 100 were males, with an average age at death of 40 years, and 54 were females, with an average age at death of 30 years and 2 months. The average duration of illness was 2½ years. Among the 60 cases of cor pulmonale, 45 had not been treated. Of these, 10 (22.2 per cent) had emphysema. In the remaining patients who had received treatment, 7 (46.6 per cent) had emphysema. Of the 154 patients without cor pulmonale, 112 had not received treatment, and among these, 13 (11.6 per cent) had emphysema. Of the remaining 42 not treated, 7 (16.6 per cent) had emphysema. Of the group of 15 treated patients, 9 had had surgery days before death: 6 pneumonectomies, 2 lobectomies, and 1 segmental resection. The findings in these were as follows: pure right ventricular dilatation, 4; pure right ventricular hypertrophy, 3; mixed lesions, 2.

Pleural complications in the form of adhesions were found in 70 (45.4 per cent) of the 154 patients without cor pulmonale, and in 28 (46.6 per cent) of the 60 with cor pulmonale. In 10 of the 60, there was pachypleuritis, with empyema in 8. In 6 of the cases of cor pulmonale, embolism or thrombosis of one of the pulmonary arterial branches was found. Of these, 4 showed right ventricular hypertrophy, 1 showed right ventricular dilatation, and 1 mixed hypertrophy and dilatation. Pneumoconiosis was found in 4 of the cases of cor pulmo-

nale. In 2 of these there was right ventricular hypertrophy, and in 2 right ventricular dilatation.

Additional general conclusions derived from this study were as follows: (*1*) Advanced, bilateral tuberculosis, with cavitation and bronchogenic or miliary spread, most often accompanies cor pulmonale. (*2*) Pleural alterations play an important role in producing obstruction to the pulmonary circulation only when very extensive and chronic, such as in empyema. (*3*) The greater incidence of cor pulmonale in treated cases is probably related to the emphysema provoked by interstitial fibrosis. (*4*) The frequency of cardiac insufficiency in cor pulmonale is directly proportional to the increase in the weight of the heart.

F. Perez Pina

Diaphragmatic Hernia (in Spanish). P. Largghero*, W. Venturino, and P. Echeverria. *Tórax*, September, 1958, 7: 228–235. (*Pasteur Hosp., Montevideo, Uruguay)

A series of 46 observations in 45 patients with nontraumatic diaphragmatic hernia was studied. The presenting clinical pictures were: gastrointestinal bleeding, 15; dysphagia, 11; pain, regurgitation, heartburn, or dyspepsia, 8; chronic secondary anemia, 2; roentgenographic shadow, 2; neoplastic syndrome in aged, 2. Surgery was performed in 35 cases, 24 by the thoracic approach, 9 by the abdominal approach, and 2 by a thoracoabdominal approach. There was 1 death from duodenal fistula on the 18th postoperative day, in a patient aged 74. The abdominal approach was used in this case.

In addition, there were: 2 recurrences, 1 from the thoracic approach series and 1 from the abdominal approach series; 1 serious complication, a recalcitrant gastroileocolic distention; 1 remote gastrointestinal bleeding in an otherwise asymptomatic patient with well-reduced hernia, cause not apparent. There were 16 patients with associated gastrointestinal disturbances (35 per cent), as follows: cholelithiasis, 6; strawberry gallbladder, 2; cystic duct stenosis, 1; gastric or duodenal ulcer, 4; cancer of the stomach, 1; bleeding gastric polyposis, 1; secondary ulcer of the esophagus with stenosis, 2; previous gastroenterostomy, 3. In this series, $\frac{2}{3}$ of the cases were females. Age at operation was more than 50 in 75 per cent. The oldest patient operated upon was 82.

The following diaphragmatic hernias should be operated upon: (*1*) Children of any age. (*2*) Patients with bleeding, dysphagia, or reflux. (*3*) Large hernias, any age. (*4*) Suspicion of associated gastric, cardiac, or esophageal lesion. (*5*) Any hernia accessible and easy to repair in the course of some other abdominal surgery. Diaphragmatic hernias of small or medium size in which proper dietary and postural treatment produce relief of symptoms and hernias—even in children—in which contraindications of age or of other organic disease exist, should not be operated on.

F. Perez Pina

Unilateral Pulmonary Embolism with Increased Compensatory Circulation through the Unoccluded Lung. F. G. Fleischner*. *Radiology*, October, 1959, 73: 591–597. (*Beth Israel Hosp., Boston, Mass.)

In massive unilateral pulmonary embolism, the entire or greater portion of the pulmonary arterial blood is shunted to the unoccluded lung. This condition may be well recognized on a technically good roentgenogram. The lung field of the embolized side is more translucent due to the oligemic capillary background; the visible individual vessels are narrower and fewer in number; the hilar shadows may be plump as the result of the widened hilar arteries, distended either by the embolothrombus or by pulmonary hypertension associated with pulmonary embolism. In the nonobstructed lung, the hilar and peripheral vessels are wider owing to the overflow of blood to this side. These changes may be transient or permanent.

Two illustrative cases are presented. It is not yet clear whether angiocardiography can be performed with impunity in instances of suspected embolism. On the other hand, electrokymography may provide a sensitive tool for the discovery of diminution or absence of pulsation and flow in smaller pulmonary areas.

W. J. Steininger

Unilateral Pulmonary "Emphysema." I. Katz* and S. Wagner. *Radiology*, September, 1959, 73: 362–366. (*V. A. Hosp., Brooklyn, N. Y.)

Under the designation of "unilateral pulmonary 'emphysema' " an additional case is reported of a unique pulmonary disease with distinctive clinical and roentgenographic features but undetermined etiology. Pulmonary function and bronchospirometric studies in this patient revealed markedly reduced ventilation and decreased oxygen consumption by the abnormal lung. The characteristic roentgen features of this disease are unilaterally decreased prominence of the pulmonary vascular shadows and a corresponding decrease in the size of the hilar shadow as contrasted with that of the opposite lung. In approximately half of the cases the mediastinum is displaced toward the affected side on inspiration. Expiratory films accentuate the radiolucency of the abnormal lung. The abnormal movements of the mediastinum and the increased airway resistance are believed to be reflections of decreased

pulmonary elastic recoil and distensibility. The clinical course is almost invariably benign. The condition should be differentiated primarily from obstructive and compensatory forms of pulmonary emphysema and congenital absence of a main branch of the pulmonary artery.

W. J. STEININGER

Fibrocystic Disease of the Pancreas Presenting as Bronchiectasis in an Adolescent. A. W. LEES* and G. B. S. ROBERTS. *Brit. J. Dis. Chest*, October, 1959, 53: 365–368. (*Ruchill Hosp., Glasgow, Scotland)

An account is given of a female who died at the age of 16 years from an exacerbation of chronic respiratory infection. During life there had been no indication of gastrointestinal disturbance, but histologic examination post mortem revealed lesions characteristic of fibrocystic disease of the pancreas in the lungs, pancreas, and liver.

Attention is drawn to the possibility of failing to recognize that fibrocystic disease of the pancreas is the underlying cause of chronic respiratory infection, especially in an older child or adult who has no gastrointestinal symptoms. Increased salt content of the sweat, present in at least 99 per cent of cases, is the most constant manifestation of the disease, and sweat analysis is therefore the most reliable diagnostic test. Fibrocystic disease of the pancreas is hereditary, and investigation of relatives of florid cases has shown that the characteristic alteration in sweat electrolytes may be the only manifestation of the disease; or, there may be a positive sweat test and chronic respiratory infection, but no evidence of pancreatic dysfunction. About 10 per cent of patients with fibrocystic disease of the pancreas die shortly after birth as a result of meconium ileus but, if this first hurdle is surmounted, prognosis depends chiefly on the degree of respiratory involvement. The outlook in fibrocystic disease of the pancreas is better than earlier experience suggested, partly because of improved therapy for respiratory infection. It is suggested that sweat analysis be carried out in all children and young adults in whom resection for bronchiectasis is contemplated, as the result may well influence the decision to operate.

M. J. SMALL

Familial Pulmonary Fibrosis and Its Relationship with the Hamman-Rich Syndrome. B. LASKI*, W. L. DONOHUE, I. UCHIDA, and J. D. MUNN. *A. M. A. J. Dis. Child.*, October, 1959, 98: 503–504. (*Hosp. for Sick Children, Toronto, Canada)

The Hamman-Rich syndrome terminated fatally in 2 siblings, both less than 1 year of age. The father died from the same disease within 1 month of the death of the second child. Further careful investigation revealed 7 members in all of this family to have been affected. In addition, another family with 2 affected members was also found. Clinically, these 2 patients both presented at 3 months of age with the symptom of "failure to thrive." Despite exhaustive investigation, nothing was found; they both reappeared a few months later with signs of progressive pulmonary disease, and died. The father was found to have pulmonary fibrosis and he, too, died of a superimposed acute pneumonia. Pathologically, they all showed the lesions typical of the Hamman-Rich syndrome, and it would seem that at least one cause of this syndrome has a genetic base.

M. J. SMALL

Congenital Broncho-Oesophageal Fistula Without Oesophageal Atresia. B. R. BILLIMORIA. *Indian J. Chest Dis.*, July, 1959, 1: 1–9.

Six cases are reported of congenital esophagobronchial fistulas which were treated by surgery in patients ranging in age from 4 months to 35 years. The embryology of the condition and some of the literature are reviewed. It is suggested that the incidence of this anomaly may be higher than is generally accepted, and its surgical significance in the treatment of bronchiectasis is discussed. Conservative treatment, consisting of closure of the fistula without pulmonary resection, may prove to be all that is required in children even in the presence of an apparently established bronchiectasis.

Hypnosis in the Treatment of Hiccups Unresponsive to Conventional Treatment. G. BENDERSKY* and M. BAREN. *A. M. A. Arch. Int. Med.*, September, 1959, 104: 417–420. (*Hahnemann Med. College and Hosp., Philadelphia, Pa.)

A case is presented of a patient with prolonged hiccups seriously complicating a myocardial infarction, with the subsequent termination of hiccups under hypnosis after failure with conventional therapy. It is suggested that in cases of "intractable hiccups," despite the elicitation of specific organic causes, the psychogenic factor may predominate and allow hypnotic resolution. Brief discussion is presented of proposed mechanisms of hiccup production with relation to hypnosis for the purpose of revealing the inadequacy of our present knowledge in this field.

W. J. STEININGER

Kartagener's Syndrome with Fibrocystic Disease. N. M. BROWN* and A. N. SMITH. *Brit. M. J.*, October 17, 1959, No. 5154: 725–728. (*Western Infirmary, Glasgow, Scotland)

A case is described of situs inversus viscerum which required appendectomy for acute obstruc-

tive appendicitis. This patient also had had repeated attacks of pulmonary infection related to bronchiectasis and sinusitis, the syndrome described by Kartagener in 1933. The patient had anomalies attributable to mucoviscidosis or fibrocystic disease—namely, abnormalities of the fat and nitrogen in the stool. He was 20 years old—unusual in patients suffering from known mucoviscidosis. The anomaly of the salt content of the sweat was found in a sibling (after authors' summary).

E. A. RILEY

Pleuropulmonary Manifestations in Lupus Erythematosus Disseminatus. J. MYHRE*. *Acta med. scandinav.*, September 30, 1959, 165: 55–60. (*University Clinic, Bergen, Norway)

Pleuropulmonary manifestations occurred in 16 of 18 cases of lupus erythematosus disseminatus. In 10 of the cases the changes were bilateral when first demonstrated and, with one exception, bilateral changes developed in the others. The pleuropulmonary affection often appears months or years after the onset of the disease, but cannot be considered a pre-terminal or terminal event; it may be the first local manifestation of the malady. In the basal portion of the lung, uneven and ill-defined opacities of varying size and density appear together with a pleural effusion of small or moderate size. The diaphragm tends to rise and the elevation is often unusually marked in proportion to the pleuropulmonary changes. "Plate-shaped" shadows of the type usually considered to be of an atelectatic nature tend to develop in the basal lung field. On improvement, the diaphragmatic elevation generally persists much as before. Although the separate components of the pleuropulmonary manifestations in lupus erythematosus disseminatus are not specific, together they constitute a clinico-roentgenographic syndrome which may be more characteristic than is generally appreciated.

E. DUNNER

Congenital Pulmonary Cystic Lymphangiectasis. J. FRANK* and P. C. PIPER. *J. A. M. A.*, October 24, 1959, 171: 1094–1098. (*Madison General Hosp., Wisconsin)

Congenital pulmonary lymphangiectasis is considered a primary developmental malformation of the lymphatic system and can be associated with lymphangiectasis in other organs. Two cases are reported, one in a full-term baby who died two hours after birth, and one in a full-term stillborn infant.

H. ABELES

Isolation of Measles Virus at Autopsy in Cases of Giant-Cell Pneumonia Without Rash. J. F. ENDERS*, K. MCCARTHY, A. MITUS, and W. J. CHEATHAM. *New England J. Med.*, October 29, 1959, 261: 875–881. (*Harvard Med. School, Boston, Mass.)

Agents identified as measles virus were isolated from materials taken at autopsy from pulmonary secretions or tissue in 3 typical cases of giant-cell pneumonia. None of these patients had signs or symptoms of typical measles during their illness. These findings afford direct evidence for the etiologic role of measles virus in giant-cell pneumonia. No satisfactory explanation for the failure of the patients with giant-cell pneumonia to present the usual manifestations of infection with measles virus is available. It is possible that measles virus was the cause of this condition, and that the normal capacity of the tissues to react is depressed by the pre-existence of chronic disease.

M. J. SMALL

Persistence of Measles Virus and Depression of Antibody Formation in Patients with Giant-Cell Pneumonia after Measles. A. MITUS*, J. F. ENDERS, J. M. CRAIG, and A. HOLLOWAY. *New England J. Med.*, October 29, 1959, 261: 882–889. (*Children's Hosp., Boston, Mass.)

From 2 patients with pneumonitis that followed the subsidence of signs of measles, agents identified as measles virus were isolated. The virus was also recovered from tissues taken at autopsy. The diagnosis of giant-cell pneumonia was made provisionally during the course of the illness and confirmed after death. Measles virus persisted for an unusually long period in the upper respiratory tracts of these patients, who also failed to respond in the normal manner by development of specific antibodies. The clinical and pathologic features of the pneumonitis were indistinguishable from those found in the patients with giant-cell pneumonia not associated with overt measles. In 2 other patients who survived pneumonitis that followed measles, measles virus was found to persist for unusually long periods in 1, and the antibody response appeared to be depressed in both. Accordingly, it is probable that giant-cell pneumonia was also present in these 2 children.

Laboratory diagnostic methods now available permit the clinical recognition of giant-cell pneumonia caused by measles virus. To make this diagnosis appears important, especially in regard to therapy and prevention. Since the 2 surviving patients in this study received large quantities of measles antibody, it is possible that seroprophylaxis and serotherapy will prove effective. The survival of 2 patients who received large doses of gamma globulin at the time of exposure suggests

that this material may be of value in modification of subsequent measles pneumonitis. It is possible that very large quantities of antibody administered intravenously in the form of measles convalescent plasma will mitigate this condition, since rapid recovery in 1 case followed this procedure. It is obvious that cases of measles pneumonitis without rash represent potential sources of infection. Therefore, to be able to recognize them as soon as possible after admission to the hospital is of importance in the interest of preventing outbreaks of measles in the wards.

M. J. SMALL

Chronic Fibrous Mediastinitis Due to *Histoplasma Capsulatum* (Histoplasmal Mediastinitis). Report of Three Cases with Different Presenting Symptoms. G. F. LULL, JR.* and D. F. WINN, JR. *Radiology*, September, 1959, 73: 367–373. (*Fitzsimons Army Hosp., Denver Colo.)

Three cases of chronic fibrous mediastinitis each presented different clinical manifestations and in each case *H. capsulatum* was identified in the surgical specimen. It is postulated that *H. capsulatum* is an important etiologic agent in the production of chronic fibrous mediastinitis and that many of the cases previously reported as being tuberculous or idiopathic were in reality due to histoplasmosis. The term "histoplasmal mediastinitis" is suggested. Extension of the mediastinal mass to the right of the sternum has prognostic value as an indication of future superior vena caval obstruction. The differential roentgen diagnosis is nonspecific. The immediately pressing problem is differentiation from bronchogenic carcinoma.

W. J. STEININGER

Acute Mercury Vapor Poisoning. A Report of Four Cases with Radiographic and Pathologic Correlation. C. T. TENG* and J. C. BRENNAN. *Radiology*, September, 1959, 73: 354–361. (*Jefferson Davis Hosp., Houston, Texas)

Four cases of acute mercury vapor poisoning in a mother and three young children are reported. Only the mother survived the intoxication. The major pathologic changes in such cases are found in the lungs. Death is attributable to loss of respiratory function as a result of severe injury of the pulmonary tissues. Pathologically, the primary lesions are: (*1*) acute exudative pulmonary edema, and (*2*) acute necrotizing bronchiolitis. Secondary to these primary reactions are: (*1*) bronchiolar obstruction of the check-valve type, (*2*) alveolar and interstitial emphysema, (*3*) tension pneumothorax, (*4*) hyaline membrane formation, (*5*) secondary bacterial pneumonitis, and (*6*) different phases of resolution. All of these changes are demonstrated in serial chest films.

W. J. STEININGER

Alveolar Echinococcosis of the Liver Metastasizing in the Lungs (in German). H. G. HAAS* and J. R. RÜTTNER. *Schweiz. med. Wchnschr.*, August 1, 1959, 89: 813–817. (*Zollikerberg, Switzerland)

A 76-year-old male died after suffering for 9 months of liver disease, eosinophilia, and metastatic pulmonary lesions. The tentative clinical diagnosis was liver cirrhosis with cancer. The autopsy revealed echinococcosis of the liver with multiple metastases scattered throughout both lungs. The diagnostic criteria thought to be typical had only a limited value in this case. The patient had never had any contact with dogs, but had kept cats. The intracutaneous test with hydatid fluid (Casoni's test) had been negative.

J. HAAPANEN

Exudative Pleurisy as a Diagnostic Problem (in Norwegian). P. HOVEID*. *Nord. med.*, September 10, 1959, 62: 1360–1363. (*Aker Sykehus, Oslo, Norway)

From 1953 to 1957, 100 cases of exudative pleurisy were treated. Of these, 21 were carcinomatous, 16 tuberculous, 22 probably tuberculous, and 9 of other infectious origin. In the differential diagnosis, the culture of the tubercle bacillus was of a limited value. Cytologic examination of the pleural fluid gave promising results in the discovery of malignant changes. In 1958, a needle biopsy of the parietal pleura was performed on 40 patients, using Abrams' needle, without any complications. Although simple, this method gives valuable information for the differential diagnosis of exudative pleurisy.

J. HAAPANEN

Studies on Primary Atypical Pneumonia. II. Observations Concerning the Development and Immunological Characteristics of Antibody in Patients. C. LIU*, M. D. EATON, and J. T. HEYL. *J. Exper. Med.*, June, 1959, 109: 545–556. (*Harvard Med. School, Boston, Mass.)

This report presents observations on: (*1*) the development and persistence of antibody in human patients with primary atypical pneumonia (PAP) infection, (*2*) the relationship of PAP antibody to "cold" and Streptococcus MG agglutinins, and (*3*) the use of the fluorescent-staining reaction as a specific serologic diagnostic test for PAP.

Infected chick-embryo lungs were used for the titration of PAP antibody in acute and convalescent serum by means of the indirect method of fluorescent staining. (*1*) From 43 patients who

had PAP antibody in their convalescent serum and for whom the time of onset of disease was known, it was found that PAP antibody developed during the second and third weeks of illness. Six of these subjects were followed for a year or longer, and antibody was still detectable 12 to 18 months after illness. (*2*) Although positive "cold" agglutinins and Streptococcus MG agglutination in human convalescent serum may substantiate the diagnosis of PAP, it was shown that the titers of these antibodies usually declined within 4 to 6, and 7 to 8 weeks after infection, respectively. Moreover, in some patients with PAP antibody, the tests for "cold" agglutinins and Streptococcus MG antibodies were negative. Cross-absorption tests on serum containing high PAP antibody titers and "cold" or MG agglutinins demonstrated that absorption with "cold" or MG agglutinins did not remove PAP antibody. Furthermore, the serum of rabbits immunized with MG organisms or with PAP-infected chick embryo lung suspensions showed no cross-reaction between the two antigens. (*3*) The sensitivity of the fluorescent-staining technique in determining PAP antibodies was indicated by detecting a rise in serum-antibody titer during convalescence in 67 to 92 per cent of the patients from 3 separate epidemics, comprising a total of 89 subjects. Absorption of the serum with chick embryo powder, chick lung powder, or mouse liver powder did not affect the PAP antibody titer.

C. H. PIERCE

Current Experiences in the Serologic Diagnosis of Primary Atypical Pneumonia and Related Illnesses with a Battery of Antigens. I. Current Status of the Diagnostic Value of Cold Hemagglutinin. I. TATENO*, A. SUGIURA, S. SUZUKI, T. MORITA, O. KITAMOTO, M. TOGASHI, K. SUZUKI, Y. KANEKO, A. KAWASHIMA, T. SATO, and S. OBARA. *Jap. J. Exper. Med.*, October, 1959, 29: 457–470. (*Hosp. of the Institute for Infectious Diseases, U. of Tokyo, Japan)

Serologic diagnoses of respiratory infections such as primary atypical pneumonia (PAP), influenza, acute respiratory disease (ARD), et cetera, were made using a battery of viral antigens and the cold hemagglutination test during the period October, 1956, through December, 1958, and the following conclusions were obtained.

(*1*) The hypothetic, cold hemagglutinin-inducing agents(s) (CHA-IA) which, upon infection in man, induces cold hemagglutinin in the blood of patients, provokes a variety of respiratory diseases including the so-called PAP, ARD, and subclinical infections. This agent(s) was apparently as widespread as the influenza A virus during this period of examination, as judged from the distribution of CHA in the blood serum of patients with various respiratory diseases. Because of this widespread prevalence of this agent(s) in the civilian population, it was concluded that single or paired serum specimens with high CHA (1 in 128 or more), but without significant rise in titer during observation, were not considered diagnostic but merely represented prior infection with this agent. (*2*) Influenza A virus was as frequent or more frequent in its distribution than CHA-IA, followed by adenovirus; infection with influenza B was rare during this period of study. (*3*) Despite the presence of high CHA in various types of respiratory diseases, such as influenza and adenovirus infections, this test was considered useful in identifying certain types of respiratory diseases when the serum specimens were appropriately timed; when simultaneous serologic tests against influenza A and B, adenoviruses, et cetera, were made; and when the final results were analyzed from the results of this battery of tests.

II. Diagnostically Complicated Cases. I. TATENO, S. SUZUKI, O. KITAMOTO, M. TOGASHI, K. SUZUKI, Y. KANEKO, S. OBARA, T. SATO, AND N. KASAI. Pp. 471–480.

Serologic diagnoses were made with a battery of antigens in cases of transient pulmonary infiltration or viral pneumonia on serum obtained at various phases of illness; it was concluded that such tests should be made on appropriately timed serum from acute and convalescent stages. So-called PAP or viral pneumonia should not be considered in cases with pulmonary infiltrations which last for more than 1 or 1½ months.

The following cases were presented to substantiate the authors' opinion, based on 2½ years' experience. (*1*) Influenza A-associated pneumonia with simultaneous increase in cold hemagglutinin. (*2*) Pulmonary tuberculosis with a significant increase in cold hemagglutinin. (*3*) Significantly "high" or "positive" cold hemagglutinin in influenzal pneumonia, pharyngoconjunctival fever, Izumi fever, paratyphoid B, and lung cancer. Cases of pneumonia which may be considered "true" and "pseudo" cold hemagglutinin-positive were also presented.

I. TATENO

Pneumocystis Pneumonia Associated with Agammaglobulinaemia. J. G. B. RUSSELL*. *Arch. Dis. Child.*, August, 1959, 34: 338–341. (*Royal Manchester Children's Hosp., Pendlebury, England)

A patient with *Pneumocystis carinii* pneumonia is described in whom there was associated agam-

maglobulinemia. The latter seemed to be congenital in origin, and is unusual in that it occurred in a female infant (Author's summary).

E. A. RILEY

Staphylococcal Pneumonia in Children: A Review of 83 Cases. R. KOCH*, M. J. CARSON, and G. DONNELL. *J. Pediat.*, October, 1959, 55: 473–480. (*U. of Southern California, Los Angeles)

The increase in incidence of staphylococcal pneumonia has been pronounced in recent years. The records of 83 patients seen between 1944 and 1958 at the Los Angeles Childrens Hospital are reviewed. Ten patients died, all but 1 were less than 4 months of age.

Bacitracin, chloramphenicol, erythromycin, oleandomycin, and novobiocin were valuable agents for treatment. Bacitracin with one other effective antimicrobial resulted in an improved clinical course and a lower mortality rate. Closed catheter drainage was used when empyema or pyopneumothorax occurred. Rib resection was necessary when drainage was inadequate; decortication of the lung was generally reserved for patients with persistent atelectasis. Long-continuing pulmonary abscess was seen in the right middle lobe in 3 patients who responded to lobectomy.

The danger of superimposed Pseudomonas infection is emphasized. Polymyxin with either streptomycin or oxytetracycline is the treatment of choice for this complication (after authors' summary).

M. J. SMALL

Spontaneous Pneumothorax in Apparently Healthy Flying Personnel. G. DERMKSIAN* and L. LAMB. *Ann. Int. Med.*, July, 1959, 51: 39–51. (*School of Aviation Med., USAF, Air U., Randolph Air Force Base, Texas)

Among flying personnel, 25 individuals who had experienced 38 episodes of spontaneous pneumothorax were studied at the School of Aviation Medicine. Following re-expansion of the involved lung, pulmonary function tests of ventilation were within normal limits in all 25 cases. The routine postero-anterior and lateral chest roentgenograms disclosed pathologic entities (cysts, emphysematous bullae, or pleural blebs) which would account for the spontaneous pneumothorax in 7 of the cases (28 per cent). In an additional 7 cases (28 per cent), apical laminagraphy revealed the pathologic etiology.

In 11 cases, roentgenographic studies were performed in the altitude chamber. In 6 of these, in which the routine roentgenographic studies were unremarkable, the chamber films were of no additional help. In 1 of the patients, however, the films taken at various altitudes demonstrated the presence of an expanding bulla in the left apex and a very small, limited pneumothorax. These findings were not demonstrable on the routine roentgenographic studies, except in retrospect. In the 4 remaining cases, in which the pulmonary pathologic process resulting in the pneumothorax had been demonstrated by roentgenographic studies, the altitude chamber films disclosed that these were air-containing cavities which expanded with increasing altitude (decreasing atmospheric pressure).

T. H. NOEHREN

Serum Proteins in Childhood Tuberculosis. C. M. ZITRIN*, E. M. LINCOLN, R. CARRETEREO, and E. MELLY. *A. M. A. J. Dis. Child.*, September, 1959, 98: 330–341. (*Bellevue Hosp., N. Y., N. Y.)

In 130 children with primary tuberculosis or complications thereof, it was found that the serum α_2- and γ-globulins were increased in proportion to the increasing severity of the tuberculous infection. The α_2-globulin was determined by means of the tryptophane-acid reaction devised by Seibert, and the γ-globulin was measured by the Kunkel zinc sulfate turbidity method. Exceptions to this occurred in all of the patients with tuberculous meningitis and in 6 of the 12 patients with miliary tuberculosis, these showing an elevated α_2- and relatively low γ-globulin. This disparity, occurring in the sickest patients, was interpreted as the worst prognostic sign.

In 33 patients followed at monthly intervals with serial studies of the globulins, the patients who were treated and had uncomplicated courses showed a gradual decrease in the two globulin fractions which appeared to parallel clinical improvement. In 5 untreated patients, 3 with primary tuberculosis and 2 with tuberculous pleurisy, the globulin fractions remained elevated much longer than in the treated patients of all degrees of disease, including most of the children with miliary tuberculosis and meningitis. Tuberculous complications in these children were followed (or accompanied) by a rise in the γ-globulin and, to a slighter degree, the α_2-globulin. A similar rise was seen following measles and chicken pox, but no increase was noted following upper respiratory infections, acute otitis, and other nontuberculous intercurrent infections.

There were no significant changes in the total protein or A/G ratio in these children, except for 2 with marked, protracted tuberculous disease.

M. J. SMALL

Clinical and Pathophysiologic Findings in Isolated Congenital Pulmonic Stenosis (in German). A. TSAKIRIS*, F. SCHAUB, and A. BÜHLMANN. *Schweiz. med. Wchnschr.*, August 1, 1959, 89: 801-810. (*U. of Zurich, Switzerland)

A report on 18 patients.

J. HAAPANEN

Partial Thoracic Stomach in Childhood. J. B. BURKE*. *Brit. M. J.*, October 24, 1959, No. 5155: 787-792. (*Hosp. for Sick Children, London, England)

The symptomatology, diagnosis, and management of 137 children with partial thoracic stomach are discussed. The main symptoms in 132 with small intrathoracic loculus of the stomach were vomiting (dating from birth in 77 per cent), hematemesis (89 per cent), dysphagia (38 per cent), and unsatisfactory gain in weight. Males predominated by 2.7:1 and were more severely affected than females. In uncomplicated cases, improvement began with introduction of solid foods, and vomiting was infrequent after the first year. The prognosis in children presenting under 1 year of age was very good in 91 per cent.

Dysphagia and narrowing of the esophagus were of serious prognostic significance. Organic fibrous contracture of the esophagus was present in 33 children, in 27 there was variable esophageal narrowing, and in 72 there was no esophageal narrowing (uncomplicated cases). Sixty-eight children were treated medically. Of the 64 children subjected to surgery, 38 had some narrowing of the esophagus. An organic stricture was found at operation in 25 cases. In 20 children surgery was performed because of severe esophagitis. In only 12 cases was there immediate response to the operation with cessation of symptoms. Eleven of the 64 children (17 per cent) appeared to derive little or no benefit from repair of the hernia. Three of these developed organic stricture after surgery.

E. A. RILEY

The Natural History of the Partial Thoracic Stomach (Hiatus Hernia) in Children. I. J. CARRÉ*. *Arch. Dis. Child.*, August, 1959, 34: 344-353. (*Queen's U., Belfast, Ireland)

A review is given from which the natural history of the partial thoracic stomach in children has been determined. Evidence is also presented indicating the changes in symptomatology on weaning to solid food provide a useful guide to a patient's further progress. It is concluded that about 60-65 per cent of all clinically affected and untreated children pursue a relatively benign clinical course, the majority being free of symptoms by 2 years of age. With but few exceptions, these children show improvement either upon, or coincident with, weaning on to solid food. Symptoms in a further 35 per cent are not improved by weaning on to a mixed diet. Most of these patients suffer from troublesome symptoms up to the age of 4 and longer. A few of these latter children, representing about 5 per cent of all patients, develop an esophageal stricture. It has been estimated that in the absence of an esophageal stricture less than 5 per cent of all untreated patients would die (after author's summary).

E. A. RILEY

Fatal Cardiovascular and Respiratory Failure Associated with Tracheotomy. N. M. GREENE*. *New England J. Med.*, October 22, 1959, 261: 846-848. (*Yale U. School of Med., New Haven, Conn.)

Five cases are presented of fatal cardiovascular and respiratory collapse which occurred immediately after tracheotomy. It is hypothesized that these were due to rapid reversal of pre-existing respiratory acidosis. Such an abrupt reversal of respiratory acidosis has been shown to produce, for reasons that are not yet clear, profound cardiovascular effects, including arrhythmias and arterial hypotension.

Suggestions are made regarding the care of the patient who requires a tracheotomy. Before the tracheotomy is performed the patient's blood pressure and pulse should be routinely determined. Also routinely, a vasopressor should be on hand ready for immediate intravenous injection, and arrangements should be made to put the patient in the Trendelenburg position if necessary. Immediately after tracheotomy and at least every ten minutes for the next hour, the blood pressure and pulse should be recorded. If hypotension develops, the patient should be placed in the Trendelenburg position and, if the hypotension persists, a vasopressor should be used. Oxygen should be administered cautiously, if at all, in such cases since the resultant high arterial oxygen tension may eliminate the role of the chemoreceptors in initiating respiration and so contribute to the apnea.

M. J. SMALL

Pulmonary Adenomatosis (in Portuguese). N. S. VIDAL*, F. ROSADO, W. WANDERLEY, C. C. L. SANTOS, and D. DE PAOLA. *Rev. serv. nac. tuberc.*, January-March, 1958, 2: 45-57. (*Sanatorio Curicica, Rio de Janeiro, Brasil)

One case is reported of alveolar cell carcinoma (pulmonary adenomatosis) with cardiac involvement in a 51-year-old male patient. This is the 11th case reported in Brazil. A review of the Brazilian literature on the subject is presented.

J. F. CARNEIRO

A Rare Type of Carcinoma of the Trachea. L. G. Whitby*. *Brit. J. Dis. Chest*, October, 1959, 53: 360–364. (*Central Middlesex Hosp., London, England)

The case history is presented of a patient who died from the effects of a large tracheal tumor. This is possibly the second recorded case of tracheal carcinosarcoma. The rarity of laryngeal and tracheal carcinoma is discussed in relation to the increasing incidence of bronchogenic carcinoma. Tobacco smoke is composed mainly of microscopic oily droplets held in suspension in air; these droplets may show less tendency to aggregate and be deposited during their rapid and turbulent passage through the larynx and trachea than the bronchial and alveolar network, where other factors such as humidification and the progressive cooling of the smoke may assume importance. This may explain the relative rarity of laryngeal and tracheal carcinoma, on the assumption that the most probable cause of bronchial carcinoma, a component of cigarette smoke, is failing to make contact with the laryngeal and tracheal epithelium in these sites.

M. J. Small

Hodgkin's Disease Associated with Other Malignant Tumors and Certain Non-Neoplastic Diseases. D. V. Razis*, H. D. Diamond, and L. F. Craver. *Am. J. M. Sc.*, September, 1959, 238: 327–335. (*Memorial Center for Cancer and Allied Diseases, N. Y., N. Y.)

The coexistence of Hodgkin's disease with other malignant neoplasms, other lymphomas, and certain nonmalignant diseases was studied in a group of 1,102 patients with Hodgkin's disease, 1,269 patients with lymphosarcoma, and 220 patients with leukemia. Other malignant neoplasms were found in 2.2 per cent of the patients with Hodgkin's disease as compared with 3.9 per cent in the lymphosarcoma group and 2.3 per cent in the leukemia group. Lymphosarcoma was associated with Hodgkin's disease in 2 patients, and in 2 others Hodgkin's disease was associated with monocytic leukemia. In contrast, 7.6 per cent of the lymphosarcoma patients developed leukemia. Active tuberculosis was associated with Hodgkin's disease more often (1.8 per cent) than with lymphosarcoma (0.3 per cent), and this difference was highly significant statistically. The incidence of active tuberculosis in the leukemia group was 1.4 per cent. Secondary amyloidosis was found in 5 patients with Hodgkin's disease but in no patients with lymphosarcoma and leukemia. The association is reported of Hodgkin's disease with Boeck's sarcoid, the so-called "collagen diseases," idiopathic thrombocytopenic purpura, diabetes mellitus, and syphilis.

W. J. Steininger

Extraosseous Infiltration in Multiple Myeloma. J. A. Gilroy* and A. B. Adams. *Radiology*, September, 1959, 73: 406–409. (*U. of Rochester School of Med., N. Y.)

In the bizarre disease, multiple myeloma, the possibility of soft-tissue lesions is often overlooked. A reported case is noteworthy because of extensive myelomatous involvement throughout the body with but a single bone lesion, a rounded osteolytic area in the skull. In addition there was a pulmonary lesion that simulated a bronchogenic carcinoma or a lymphoma roentgenographically.

W. J. Steininger

Mediastinal Neurofibrosarcoma Causing Hypoglycemia. E. M. Rossman*. *A. M. A. Arch. Int. Med.*, October, 1959, 104: 640–642. (*1902 W. Addison St., Chicago, Ill.)

A case is presented of mediastinal neurofibrosarcoma associated with severe hypoglycemia of the fasting cerebral type. Removal of this neoplasm was accompanied by complete disappearance of all of the neurologic symptoms and the return of the blood sugar level to normal. It is believed that there is reason to assume that those fibrosarcomas capable of producing hypoglycemia originate from the sympathetic nervous system (Author's summary).

W. J. Steininger

A Case of Multiple Tuberculoma of the Lung. J. N. Mäkipaja*. *Acta tuberc. scandinav.*, No. 4, 1959, 37: 349–351. (*Central Sanatorium of Middle Finland, Kinkomaa)

Roentgen examination of the lungs of a 40-year-old male revealed 20 round shadows 1- to 8.5 cm. in diameter which, at autopsy, were found to be typical tuberculomas, 21 in number. The round shadows, some of which exhibited areas of cavitation, increased in size and number in the course of a few years and were not influenced by antituberculosis therapy.

W. J. Steininger

TUBERCULOSIS, PULMONARY

Tuberculosis in Childhood. Part V. Histological Observations Concerning the Presence of Tubercle Bacilli in Lymphonodogenic Regressive Chronic Pneumonia of Tubercular Origin. P. Schwartz*. *Arch. Pediat.*, September, 1959, 76: 350–359. (*Warren State Hosp., Pennsylvania)

There is no regressive inflammatory pulmonary condensation in the course of a tuberculous

disease developed independently of tubercle bacilli. Therefore, the concept of an "epituberculosis," as defined 25 years ago and still widely accepted, remains without foundation. Also, it seems misleading to employ this term in the future for cases in which, following intrathoracic lymph node tuberculosis, the collapse of pulmonary parts occurs. The designation "lymphonodogenic atelectasis" appears to be more correct. Indeed, the fact should always be remembered that "pure" atelectasis by lymphonodogenic compression is an exceptional phenomenon in tuberculosis, while aspiration-infiltrates predominate.

M. J. SMALL

The Ultimate Fate of Calcified Tuberculous Lesions in Children and Adolescents (in German). H. D. RENOVANZ*. *Tuberkulosearzt*, August, 1959, 13: 519–528. (*Klinik Aprath, Rheinland, Germany)

Calcified tuberculous lesions were found in 532 of 5,216 children (10.2 per cent). The average interval between the first roentgenographic manifestation of tuberculosis and roentgenographic evidence of calcification was 11.2 months for children under 2 years of age, and 18 to 22 months in older children. In 1.8 per cent of the cases a resolution of the calcified lesions was observed; this occurred partly by absorption, and partly by softening followed by expectoration of the caseated mass. Exacerbation of the tuberculous process was observed in 13 per cent of the children with calcified lesions. In 17 per cent of these cases it was associated with cavitation.

A comparison of these figures with those calculated for adults showed that the risk of calcified foci becoming active and undergoing cavitation increases after puberty. The present observations indicate that a calcified focus does not invariably represent the last stage in the course of the tuberculous disease. It often constitutes a reservoir of tubercle bacilli which may at any time resume growth and cause reactivation of the tuberculous process.

J. HAAPANEN

Leukemia and Tuberculosis. C. LOWTHER*. *Ann. Int. Med.*, July, 1959, 51: 52–56. (*Western General Hosp., Edinburgh, Scotland)

Post-mortem reports of 27,104 cases were reviewed, and tuberculosis was found in 6.5 per cent. Tuberculosis was found in 5.6 per cent of 285 post-mortem reports on cases of leukemia. It is concluded that tuberculosis occurs infrequently in association with leukemia, and no more often with leukemia than in post-mortem material generally.

T. H. NOEHREN

Disturbances of the Pulmonary Circulation in Tuberculosis (in German). H. G. SCHOLTZE*, E. BETZ, and H. HUNDESHAGEN. *Tuberkulosearzt*, September, 1959, 13: 595–605. (*Sanatorium Sonnenblick, Marburg, Germany)

Selective angiography, lung function tests, and determination of the circulation time were performed on 50 patients with pulmonary tuberculosis with the view of obtaining information on the location and the extent of the lung lesions. By injecting I^{131}-labeled albumin through a heart catheter into the pulmonary artery or its branches, the volume and the velocity of the blood flow through the whole lung and through the individual lobes or segments were determined, in a few cases before as well as after the operation. The method proved to be valuable in assessing the indications for thoracic surgery.

J. HAAPANEN

Recent Trends in the Treatment of Tuberculosis. J. L. BLAISDELL*, H. S. COULTHARD, G. L. GALE, D. R. GARRETT, and C. A. WICKS. *Brit. J. Dis. Chest*, October, 1959, 53: 420–423. (*Toronto Hosp., Canada)

The 55th report of the Toronto Hospital surveys recent trends in their treatment of pulmonary tuberculosis. Throughout 1958, the policy of using 3 drugs concurrently (streptomycin, PAS, and isoniazid), unless otherwise indicated, was continued. This regimen has been in use since 1952 and has reduced the number of drug failures to a very small figure. Its value was indicated when a group of patients was treated on 2 drugs alone, Na PAS and a hydrazide of isoniazid, between June, 1957 and December, 1958. The rate of recovery was delayed in this group and the need for surgery of residual lesions was much higher than in the other patients who were on triple chemotherapy.

The usual dosage in use is 300 mg. of isoniazid daily, 14 gm. of Na PAS daily, and streptomycin sulphate, 1 gm. twice a week. Many forms of PAS are in use in order to obtain patient acceptance of this rather unpalatable substance. Despite the fact that calcium B-PAS does not give tissue levels or urinary levels of PAS comparable to those obtained from sodium PAS, it is unhesitatingly used in cases of intolerance of sodium PAS, and isoniazid resistance has not resulted. Patients who show a low urinary excretion of free isoniazid are given an increase of dose up to 600 or 900 mg. daily to obtain higher levels. Patients who show signs of peripheral neuritis or mental excitation are treated by substituting Compound 377 (a hydrazide of isoniazid) for isoniazid itself, and are able to continue on this form of therapy without necessarily having recourse to pyridoxine.

After 3 years of trial of cycloserine in "treat-

ment failures," it is concluded that its usefulness is very limited. It is given in the dosage of 0.25 gm. 4 times a day, and great care must be taken to space the dosage evenly or convulsions may occur. Eight cases received this in 1958, but none showed any marked benefit. The results of treating florid and fulminating cases of pulmonary tuberculosis with steroids were very satisfactory. The "average" total duration of chemotherapy (in sanatorium and post-sanatorium) is approximately 18 months to 2 years. Six hundred and forty-eight patients with pulmonary tuberculosis who had completed 1 year (or more) of chemotherapy in sanatorium were followed for at least 2 years and a maximum of 4½ years. In this time, 20 patients (3.1 per cent) had relapses.

Because of the efficacy of long-term chemotherapy in treating reversible pulmonary tuberculosis, temporary collapse has not been used for some years. No pneumothoraces have been established in the past 5 years, and tuberculous empyema has accordingly become a rarity. Therapeutic pneumoperitoneum has not been attempted in the past 3 years as it appeared to add little benefit to chemotherapy alone. Excision therapy continues to be used with only slightly reduced frequency. The main indications for resection are large areas of irreversibly destroyed lung, persistent cavities, large nodules of caseation, and bronchostenosis. In 1958 there were no standard thoracoplasties.

During the past year, an increasing number of cases of malignant disease have been recognized through laboratory investigation. Exfoliative cytology has proved of great value in the examination of sputum, bronchial secretions, and body fluids. The technique of fixation in Bouin's solution, paraffin sectioning, and staining with hematoxylin and eosin is preferred to the Papanicolaou method. This procedure can be recommended also for the recognition of pathogenic yeasts and fungi and, during the past year, established the diagnosis in a case of unsuspected pulmonary blastomycosis when other methods had failed.

M. J. SMALL

Observations on Lower Lobe Tuberculosis. J. H. PRATT-JOHNSON*. *Brit. J. Dis. Chest*, October, 1959, 53: 385–389. (*Tottenham Chest Clinic, London, N. 15, England)

The literature relating to lower lobe tuberculosis is reviewed and a further series of 30 patients reported. The evidence presented supports the theory that lower lobe tuberculosis is a manifestation of progressive primary tuberculosis in young adults and is caused by ulceration of a lower lobe bronchus by caseous mediastinal glands (Author's summary).

M. J. SMALL

Pulmonary Tuberculosis and Bronchogenic Carcinoma (in German). H. HACKL*. *Tuberkulosearzt*, September, 1959, 13: 643–646. (*Baumgartnerhöhe 1, Wien 14, Austria)

The autopsy findings in 69 cases of bronchial carcinoma associated with pulmonary tuberculosis showed that the incidence of carcinoma was not significantly higher in a lobe showing a tuberculous lesion than in other parts of the lung. The occurrence of acute pulmonary tuberculosis in patients with bronchogenic carcinoma is probably due to the lowered resistance of these cases to bacterial infection. Three cases are described in which the neoplasm was located in a tuberculous cavity, and one case in which a necrotic carcinomatous lesion was infected by *M. tuberculosis*.

J. HAAPANEN

Treatment of Patients with "Open" Pulmonary Tuberculosis with Intermittent Positive Pressure Breathing (IPPB) Apparatus (in Japanese). Y. KONDO*. *Clin. Resp. Organs*, October, 1959, 14: 799–804. (*Tokyo Municipal Fuchu Hosp., Japan)

Isoniazid and Na bicarbonate, or isoniazid and streptomycin, were given to patients with "open" tuberculosis for 3 to 5 months using the IPPB apparatus. All of these cases had been unsuccessfully treated either internally or surgically. Tubercle bacilli became negative in 8 cases and decreased in number in 5 cases. Two cases of the isoniazid–Na bicarbonate group had isoniazid-resistant tubercle bacilli before the IPPB therapy. This therapy also had a beneficial effect on subjective symptoms; shortness of breath improved in 7 cases and sputum output decreased in 12 cases, although increased sputum output was experienced in 7 cases and bloody sputum was seen in 6 cases.

The results appeared to be better the more frequently the IPPB therapy was used and the more antituberculosis drugs were used. This treatment may be useful in chronic open cases if its merits and faults are taken into consideration by the physicians who use it.

I. TATENO

The Treatment of Pulmonary Tuberculosis With Prednisolone (in German). U. BALDAMUS* and E. G. EWERT. *Deutsche med. Wchnschr.*, September 4, 1959, 84: 1625–1632. (*Dr. Heim-Krankenhaus, Berlin-Buch, Germany)

Prednisolone was given to 85 patients with extensive, cavitary pulmonary tuberculosis for an average period of 3 months, in addition to the routine chemotherapy. The initial daily dose was 20 mg., followed by a maintenance dose of 5 to 10 mg. It was used in: (*1*) hematogenous, bilateral, untreated cases with high fever; (*2*) therapy-resis-

tant, extensive, and predominantly infiltrative cavitary tuberculosis; and (*3*) tuberculous pleurisy. In ⅔ of the patients there was a definite prednisolone effect, which could be distinguished from the effects of the chemotherapy. Prednisolone was most effective in the toxic forms of tuberculosis. There was no evidence that the bacterial resistance to antituberculosis drugs was influenced by the hormone. In 6 patients prednisolone treatment made surgical intervention possible. The prednisolone was well tolerated by all of the patients. There was a striking lack of prednisolone effect in patients who had other conditions besides pulmonary tuberculosis, such as cardiopulmonary impairment, bronchial asthma, or bacterial resistance.

J. Haapanen

Studies on Serum Complement and Properdin Levels in Patients with Pulmonary Tuberculosis (in Japanese). T. Osawa*. *Kekkaku*, October, 1959, 34: 711–719. (*School of Med., Gumma U., Maebashi, Japan)

The serum complement and properdin† levels were studied in 118 patients with pulmonary tuberculosis and the following conclusions were drawn. (*1*) Serum complement levels were normal in cases with minimal lesions, low in moderately advanced, and elevated in far advanced cases. The C'_1, C'_2, and C'_3 levels were within normal limits in most cases, and only C'_4 levels were responsible for the low or elevated complement content of the patient. (*2*) Accelerated erythrocyte sedimentation rate and high complement level were usually associated with each other. Treatment with streptomycin or the bacteriologic findings in the sputum had no relation with the serum complement levels. (*3*) Complement levels rose rapidly following surgery, reaching the maximal levels in about a week, and then declined to the preoperative level in 3 weeks. The C'_4 component was responsible for the changing complement levels in this case also. (*4*) Serum properdin levels were usually low irrespective of the types of pulmonary lesions. (†*Ed. note:* Properdin is a new serum factor thought to be important in resistance to infection.)

I. Tateno

Seasonal Differences in the Serum Electrolytes of Patients with Pulmonary Tuberculosis (in Japanese). K. Hayashi*. *Kekkaku*, November, 1959, 34: 767–771. (*Nagoya U. School of Med., Japan)

The serum natrium level in patients with pulmonary tuberculosis was highest in the summer and lowest in the winter, but the levels were within normal limits. The reverse relationship was true with serum potassium, although these levels were slightly higher than in healthy subjects. Serum calcium levels were highest in autumn and lowest in winter. Changes in the seasonal levels of serum Na, K, and Ca were, however, of the same pattern as that found in healthy subjects. With regard to the yearly average levels of serum electrolytes, K was slightly higher in moderately and far advanced cases than in minimal cases, but the Na and Ca levels were the same irrespective of the type of pulmonary lesions. The seasonal difference of Na and K levels was larger in minimal cases than in other cases. In far advanced cases, Na and Ca levels were high, and K levels were low in the spring; the seasonal difference of electrolytes was most remarkable in these patients in the spring.

I. Tateno

Broncholithiasis in a Case of Active Pulmonary Tuberculosis. C. V. Patel* and A. L. Anand. *Brit. J. Dis. Chest*, October, 1959, 53: 405–408. (*K. J. Mehta Tuberculosis Hosp., Amargadh, India)

A case is presented of broncholithiasis occurring in active pulmonary tuberculosis. In this case, collapsing the cavity by first-stage thoracoplasty probably caused the broncholiths to lacerate a blood vessel, thereby producing hemoptysis and disseminating the disease to previously uninvolved lobes. The therapeutic management of patients with broncholiths differs from that of patients with ordinary cavitary tuberculosis. In such cases, techniques of pulmonary collapse are unsound because the broncholith acts like a foreign body lying in the infected space and interferes with the natural healing process. Excision of the lobe or pneumonectomy is likely to yield better results.

M. J. Small

Spontaneous Laryngeal Paralysis in Chronic Pulmonary Tuberculosis. S. K. Gupta*. *Brit. J. Dis. Chest*, October, 1959, 53: 400–404. (*Whittington Hosp., London, England)

Five cases of paralysis of the left recurrent laryngeal nerve are described. All of these patients had chronic pulmonary tuberculosis. The possible mechanisms of this association are discussed and special stress is placed on chronic mediastinitis. The literature is reviewed, and it is suggested that this syndrome may be much more common than hitherto believed. In the chronic, unilateral paralysis of the recurrent laryngeal nerves, the voice may appear entirely normal. Since temporary hoarseness of voice is a very common condition, the acute stage may often pass completely unnoticed. The condition may be missed during a routine bronchoscopy (the operator being unaware

of the condition and the cord having moved to the paramedian position). Unless patients with chronic pulmonary tuberculosis are routinely laryngoscoped, the true incidence of the syndrome will remain unknown.

M. J. SMALL

Edward VI: A Royal Consumptive. A. MACNALTY. *Brit. J. Dis. Chest*, October, 1959, 53: 369–371.

A brief account of the times and illness of this monarch.

M. J. SMALL

TUBERCULOSIS, NONPULMONARY

Treatment of Tuberculous Meningitis. W. M. FYFE*. *Arch. Dis. Child.*, August, 1959, 34: 334–338. (*U. of Glasgow, Scotland)

Forty cases of tuberculous meningitis are reviewed. All of them were treated with intramuscular streptomycin and oral isoniazid; 20 were also given intrathecal streptomycin in a minimum of 5 and a maximum of 36 injections (Group II). The results of treatment were as good without intrathecal therapy as with it. None of the children treated without intrathecal therapy (Group I) had a relapse of the disease. Seventeen patients in Group I and 15 in Group II survived. Fourteen of the survivors in each group are well without sequelae. Five patients in Group I, 3 of whom received less than 6 months' treatment, relapsed. In both groups, patients with advanced disease either died or had residual disabilities, whereas the majority (74 per cent in Group I and 82 per cent in Group II) of patients in the early and medium stages made complete recoveries. With one exception, the deaths and disabilities were confined to children less than 5 years of age. In neither group did an associated miliary tuberculosis adversely affect the prognosis, and all of the children with this association survived.

E. A. RILEY

Spinal Sarcoidosis. E. H. WOOD* and C. A. BREAM. *Radiology*, August, 1959, 73: 226–233. (*U. of North Carolina, Chapel Hill)

In sarcoidosis, involvement of the spinal cord and its membranes occurs infrequently. In 4 previously verified cases of spinal sarcoidosis, diagnosis was made on pathologic evidence after operation or necropsy. A new case is reported of sarcoidosis which, 9 months after clinical manifestation in other parts of the body, resulted in an arachnoiditis with mechanical effects on the spinal cord. Myelography was used to establish the existence, location, and extent of meningeal involvement. After operative division of adhesions and the administration of cortisone, the patient experienced almost complete functional recovery and has remained well for more than 2 years.

W. J. STEININGER

Miliary Tuberculosis with Leukemoid Reaction. Report of an Autopsied Case (in Japanese). K. SOMEYA*, Y. KOBAYASHI, Y. ISOGAI, and M. SUGIYAMA. *Jap. J. Clin. Tuberc.*, October, 1959, 18: 720–724. (*Jikei Med. School, Tokyo, Japan)

A case report is presented of a 17-year-old boy with leukemoid reaction during life who died of miliary tuberculosis. His course was characterized by high remittent fever of about 40 days' duration and hyperchromic anemia. The erythrocyte count was 1.2 million initially, and the leukocytes numbered 13,200 to 11,600 per cu. mm. Differential cell counts of peripheral blood were: myeloblasts, 24.5 per cent; promyelocytes, 49.5 per cent; myelocytes, 4.5 per cent; metamyelocytes, 1 per cent; band forms, 3 per cent; segmented leukocytes, 4.0 per cent (all of the cells mentioned above except the first are neutrophils); and lymphocytes, 13.5 per cent. There were 4 normoblasts per 200 leukocytes. Bone marrow blood had 93,000 nucleated cells per cu. mm. and the myelocytic/erythrocytic ratio was 7 to 1. The patient was given 50 mg. of prednisolone for 3 days before his death.

Numerous tubercle bacilli were found in the bone marrow, lymph nodes, and lungs where tuberculous lesions were predominant at autopsy. Cellular infiltration was present not only in the bone marrow but also in the liver, spleen, and lymph nodes, but its degree was less than in leukemia. However, it was considered that, although the differentiation of leukemia and leukemoid reaction may be possible theoretically, it may not always be easy in practice.

I. TATENO

THORACIC SURGERY

Ventricular Aneurysm or Diverticulum and Its Surgical Treatment (in German). E. DERRA* and F. LOOGEN. *Deutsche med. Wchnschr.*, September 4, 1959, 84: 1585–1590. (*Medizinische Akademie, Düsseldorf, Germany)

The successful surgical treatment of ventricular aneurysm or diverticulum in 3 children is described. In a 9-year-old boy the myocardial defect was closed with a purse-string suture placed in the superficial myocardial layers, and the aneurysmal wall sutured over it. In the other 2 cases (an 8-year-old and a 17-year-old boy) the aneurysm was resected and the myocardial defect closed with multilayered sutures under hypothermia. In the older boy the aneurysm of the right ventricle had developed after a previous transventricular pulmonary valvulotomy for pulmonic stenosis. Roentgenographic examinations, including angio-

cardiogram, later showed some myocardial bulging in the first case, but normal ventricular movements in the other 2.

J. HAAPANEN

Mediastinoscopy: A Method for Inspection and Tissue Biopsy in the Superior Mediastinum. E. CARLENS*. *Dis. Chest*, October, 1959, 36: 343–352. (*Karolinska Sjukhuset, Stockholm, Sweden)

A procedure has been sought that is simpler for the patient than thoracotomy in determining the extent to which a bronchoscopically demonstrated widening and fixation of the carina, or a roentgenographically visualized broadening of the mediastinum, is caused by metastatic carcinoma. A method is described by which it is possible, through a short incision in the suprasternal notch, to dissect down along the whole length of the trachea and to palpate with a finger the immediately surrounding tissues. A specially designed instrument, resembling a children's esophagoscope, is introduced into the passage thus dissected, and specimens from pathologic changes are excised or the tissues punctured with visual aid. The operation, which is performed under intubation anesthesia, has been used in more than 100 cases without a complication.

E. A. ROUFF

Pneumonectomy in Friedländer's Pneumonia with Abscess Formation (in German). W. HESS*. *Schweiz. med. Wchnschr.*, September 19, 1959, 89: 983–985. (*U. of Alexandria, Egypt)

A case is described of Friedländer's pneumonia with abscess formation. It is pointed out that this is an absolute indication for pneumonectomy or lobectomy.

J. HAAPANEN

Pericarditis Following Pneumonectomy for Pulmonary Tuberculosis. P. DEROOVER* and A. LACQUET. *Brit. J. Dis. Chest*, October, 1959, 53: 356–359. (*St. Raphael's Surgical U. Clinic, Louvain, Belgium)

The occurrence of an exudative pericarditis following a pneumonectomy for pulmonary tuberculosis has been mentioned rarely in the literature. Such a case is reported and the literature on the subject reviewed. Before admitting the diagnosis of cardiac decompensation after a pneumonectomy for pulmonary tuberculosis, one should first rule out the possibility of an exudative pericarditis. Although common, this complication may occur early, but also a very long time after the operation. Pericarditis should be suspected whenever the patient has a prolonged period of high fever after the operation. The negative T-wave may be absent for a long time from the electrocardiogram, and the roentgenogram may be difficult to interpret. A postoperative effusion in the thorax can easily conceal an enlargement of the heart shadow. The prognosis is poor, but on several occasions pericardial punctures have brought about a complete cure.

M. J. SMALL

Thoracoplasty and Pulmonary Resection in the Treatment of Tuberculosis (in Portuguese). A. NEVES*, S. R. BARBOSA, N. M. GUIMARÃES, and A. A. R. SILVA-NETTO. *Tisiol. pneumol.*, No. 1, 1958, 3: 93–103. (*Instituto de Tisiologia, Universidade do Brasil, Rio de Janeiro)

Based on 174 thoracoplasties *ad modum* Alexander and 158 pulmonary resections, a comparison is made of the therapeutic value of both methods. Resection restored to health without demonstrable tubercle bacilli 74.6 per cent of the cases, and thoracoplasty did the same in 62.6 per cent.

J. F. CARNEIRO

Tracheal Fenestration: A New Treatment for Chronic Pulmonary Disease. E. E. ROCKEY* and E. WASSERMAN. *A. M. A. J. Dis. Child.*, October, 1959, 98: 531. (*New York Med. College, N. Y., N. Y.)

In the treatment of chronic pulmonary diseases, the removal of thick tenacious secretions from the tracheobronchial tree often presents a difficult problem. Tracheal fenestration is a surgically created, skin-lined communication from the trachea to the anterior surface of the lower portion of the neck. The skin orifice is provided with two door-like skin valves which make the communication airtight unless the valves are pulled apart. The valves can be opened manually for the purpose of administering medication or for repeated tracheobronchial aspirations.

Tracheal fenestration was performed on 24 adult patients. All of these patients were confined to bed or home because of very severe respiratory insufficiency. Reported tracheobronchial aspirations (as many as 50 or more times per day) following the operation resulted in the removal of copious amounts of secretions. Some improvement was apparent in all patients, and 8 were rehabilitated sufficiently to permit them to be gainfully employed or to resume full household duties. Tracheal fenestration should be studied further in cases of severe progressive peripheral airway obstruction associated with retained viscid secretions.

M. J. SMALL

Permanent Tracheostomy in the Management of Pulmonary Insufficiency. E. MAYER* and I. RAPPAPORT. *Indian J. Chest Dis.*, July, 1959, 1: 10–16. (*Postgraduate Med. Center, New York U., N. Y.)

The authors distinguish three clinical phases in the evaluation of pulmonary insufficiency. Each phase requires different therapeutic management. Tracheostomy with suctioning is considered the best method to bring patients with advanced pulmonary insufficiency out of hypoventilation crisis.

PUBLIC HEALTH AND EPIDEMIOLOGY

Indiscriminate BCG Vaccination (in Portuguese). A. IBIAPINA* and E. CHEDID. *Tisiol. pneumol.*, No. 1, 1958, 3: 51–69. (*Instituto de Tisiologia, Universidade do Brasil, Rio de Janeiro)

Indiscriminate BCG, viz., BCG without previous tuberculin testing, was given by mouth to 3,487 University students, and the incidence of tuberculosis in this group was compared with a group of 2,542 nonvaccinated University students. All cases in both groups had normal roentgenograms at the beginning of the study. Cases with residual or fibrotic lesions or calcification in the lung were excluded. The follow-up was from 1 to 4 years, totaling 7,606 year-persons for the vaccinated and 4,284 year-persons for the nonvaccinated. The protection afforded by the vaccination, as it was used in this experiment, was nil.

J. F. CARNEIRO

The Value of BCG Vaccination (in German). F. HEISIG*. *Deutsche med. Wchnschr.*, September 4, 1959, 84: 1620–1625. (*Berliner Allee 56, Düsseldorf, Germany)

The beneficial effects of BCG vaccination are once again pointed out, based on statistics from many countries. The duration of the protective effect is limited: it gradually decreases after 3 years. Most striking is the reduction in the incidence of tuberculous meningitis among vaccinated persons. Even the previously tuberculin-negative nonvaccinated persons showed a 7-times higher incidence of tuberculosis than the vaccinated group.

J. HAAPANEN

A Study of the Incidence of Bronchitis Among Some Foundrymen in Derbyshire and Nottinghamshire, Compared with a Contrasting Occupational Group, and a Geographical Study of Bronchitis Mortality in the Foundry Workers of England and Wales. G. F. KEATINGE*, E. R. BUXTON, and P. J. HUNT. *Brit. J. Dis. Chest*, October, 1959, 53: 333–345. (*Butterley Co., Ltd., Ripley, Derbyshire, England)

Morbidity and mortality due to bronchitis have been studied among a group of foundrymen representing workers exposed to dust, heat, and fume, compared to a group of wagon builders whose work is done in an equable, clear atmosphere. Only men of 10 years' or more service have been included in the survey. Little evidence was found to support the generally held belief that bronchitis is a common condition in foundrymen. A geographic study of bronchitis mortality in foundry workers of England and Wales was also carried out. It is estimated that 57 per cent of foundry workers live in areas where there is an excessive death rate due to bronchitis, and it is suggested that geographic situation rather than occupation is the cause of the high bronchitis mortality returned for foundry workers and their wives.

M. J. SMALL

Lung Cancer Among White South Africans. G. DEAN*. *Brit. M. J.*, October 31, 1959, No. 5156: 852–857. (*Provincial Hosp., Port Elizabeth, South Africa)

Analysis of the 1947–1956 male lung cancer deaths in South Africa by age, country of birth, and place of residence has shown that, among those dying at age 45 to 64 (but not among those dying at age 65 and older), British immigrants have had much higher lung cancer mortality rates than Union-born men or immigrants from other countries. Further, among all 3 categories in South Africa—Union-born men, British male immigrants, and male immigrants from other countries—the lung cancer mortality rates have increased approximately in ratio to the level of urbanization and industrialization. Neither of these groups nor the urban/rural gradient can be attributed to differences in smoking habits. Instead, both would seem to have been due to the exposure of the men concerned to different degrees of atmospheric pollution. The excess lung cancer mortality among British immigrants aged 45–64 would seem to have been due to their exposure in Britain to some form of atmospheric pollution to which those emigrating before 1910 had not been subjected. The urban/rural lung cancer mortality gradient in South Africa would appear to reflect the increasing atmospheric pollution that is encountered in passing from rural areas to areas of increasing industrialization (Author's summary).

E. A. RILEY

The Prevalence of Pneumococcal Types and the Continuing Importance of Pneumococcal Infection. R. AUSTRIAN*. *Am. J. M. Sc.*, August, 1959, 238: 133–139. (*State U. of N. Y. College of Med., N. Y., N. Y.)

During the 5-year period from 1952–1957 on a general medical ward service, pneumococci were recovered from 1,322 patients, of whom approxi-

mately ⅔ had pneumonia. Of the strains isolated, 48.8 per cent were included in capsular types I to VIII inclusive, and ⅔ of 209 pneumococcal bacteremias were caused by organisms of these same capsular types. The observations reflect the continuing importance of pneumococcus as a cause of disease in man. They point also to a need for the reintroduction of capsular typing serum, and of satisfactory laboratory techniques for the isolation and identification of pneumococcus if an accurate understanding of the causes of pneumonia is to be realized.

W. J. STEININGER

Domiciliary Treatment of Pulmonary Tuberculosis in a Rural Area: A Preliminary Report on 7,858 Cases Treated in the Transkeian Territories. J. F. TAUTE* and C. J. RABIE. *Brit. J. Dis. Chest*, October, 1959, 53: 372–384. (*Tembuland Hosp., Umtata, South Africa)

The Transkei is an area in the northeastern Cape Province. The population comprises 1.6 million Bantu and 18,000 Europeans. In this area, 16,000 cases of pulmonary tuberculosis were diagnosed during a 2-year period. Domiciliary treatment of tuberculosis was instituted in September, 1955; at that time, the estimated number with active pulmonary tuberculosis was 25,000 to 30,000. The standard treatment consisted of one weekly injection of 2 gm. of streptomycin, and 400 mg. of isoniazid daily. In April, 1957, the dose of isoniazid was doubled and fixed at 10 to 12 mg. per kg. daily.

A very high percentage of patients have advanced lesions, nearly half being predominantly fibrotic lesions. The attendance for treatment is rather poor, and a disconcertingly large number of patients fail to complete their treatment. Patients with recent disease do well on treatment; the fibrotic group show but little clearing. Sputum conversion of those with advanced lesions is unsatisfactory. On the strength of available information, it is not possible to predict the effect of the present approach on the tuberculosis problem in the Transkeian Territories.

M. J. SMALL

Tuberculosis in the Family. A. F. HUSTON* and C. O. EDWARDS. *Brit. M. J.*, October 24, 1959, No. 5155: 798–801. (*Kingston Hosp. Group, Surrey, England)

During the period 1951–1958, a series of 87 tuberculous children were seen in the pediatric department of the Kingston Hospital Group: 62 had primary disease and 25 had various forms of disseminated disease. A likely source of infection was traced in 74 of the 87 children: 68 appeared to have been infected by 59 tuberculous adults in contact with them—36 by parents; 22 by grandparents, uncles, aunts, and siblings; and 10 by adult contacts outside of the family; 6 children were probably infected by tuberculous milk. No source of infection was found in 13 cases.

Comparison of the periods 1951–1954 and 1955–1958 shows that the incidence of disseminated tuberculous disease in children fell from 17 to 8 cases; the incidence of prolonged contact between tuberculous adults and children fell from 34 to 11; and the incidence of an unknown source of infection in a child fell from 10 to 3 cases. These observations emphasize the continuing need to regard tuberculosis as a serious problem, and to realize the danger to children of the tuberculous adult (after authors' summary).

E. A. RILEY

Rehabilitation Centers for Tuberculous Patients in Finland (in German). J. PÄTIÄLÄ*. *Tuberkulosearzt*, September, 1959, 13: 647–650. (*U. of Helsinki, Finland)

A description is given of three schools founded especially for the rehabilitation of tuberculous convalescents.

J. HAAPANEN

The Role of Tuberculin in the Detection of Tuberculous Infection. Editorial. *J. Pediat.*, October, 1959, 55: 541–544.

To the less exacting physician, the apparent need for multiple skin tests with different testing mediums may be sufficient to lessen his use of the tuberculin test. The more careful physician will welcome any feature which adds to diagnostic precision. It is clear that there is need for further experience with antigens from bacteria such as the nonphotochromogenic mycobacteria which may produce cross reactions with the antigen of the tubercle bacillus. To this end, it would seem wise to make available to a number of pediatric clinics the "Battey" type of antigen for skin-testing purposes, for simultaneous use with PPD, in order to extend the experience in individual children and especially in those with the smaller, questionably positive, reactions. Thus, additional information would be provided concerning possible variations in the geographic distributions of these infectious agents whose antigens produce cross reactions with tuberculin, just as there are areal distributions of the mycotic agents of histoplasmosis and coccidioidomycosis.

M. J. SMALL

Experimental and Epidemiologic Basis for the Interpretation of Tuberculin Sensitivity. C. E. PALMER*, L. B. EDWARDS, L. HOPWOOD, and P. Q. EDWARDS. *J. Pediat.*, October, 1959, 55: 413–429. (*Public Health Service, Washington, D. C.)

Epidemiologic evidence that nontuberculous infections cause many of the positive tuberculin reactions observed in human beings is corroborated by results of experimental studies of guinea pigs reported in the present paper. Some of the animals were infected with virulent tubercle bacilli, some with the nonphotochromogenic mycobacteria of the "Battey" type, and some were not infected. Intracutaneous tests with standard mammalian tuberculin (PPD-S) and the PPD antigen prepared from the Battey organism (PPD-B) showed that, although cross reactions occurred with both types of infection, the homologous antigen usually produced a larger reaction than the heterologous antigen. Frequency histograms of the sizes of reactions to PPD-S for theoretic populations containing various proportions of tuberculosis infected, Battey infected, and uninfected guinea pigs revealed patterns of sensitivity similar to those observed in human populations.

Parallel studies of patients in tuberculosis hospitals from whom only typical tubercle bacilli or Battey-type organisms had been recovered gave results similar to those found in the guinea pigs. Studies of healthy U. S. Navy recruits from different parts of the country, and of general population groups in this country and abroad, indicate that cross reactions to tuberculin are highly prevalent in some geographic areas and much less prevalent in others; moreover, the frequency of cross reactions varies with the prevalence of sensitivity to PPD-B. Simultaneous testing with PPD-S and other PPD antigens can be expected to help determine the source of the tuberculin sensitivity, just as simultaneous testing with histoplasmin and coccidioidin helps to distinguish between the two fungous infections.

M. J. SMALL

ROENTGENOGRAPHY

Mediastinal Bronchogenic Carcinoma. H. GREENFIELD* and J. GOLD. *Am. J. Roentgenol.*, July, 1959, 82: 108–113. (*Kings County Hosp. Center, Brooklyn, N. Y.)

A bronchogenic neoplasm may often present on the initial roentgenographic examination as a large, round, and often symmetric mediastinal mass. The 3 cases presented here demonstrate the extent to and manner in which the mediastinum becomes involved. The tumor may invade the anterior, middle, or posterior mediastinum with compression of the vital structures. Roentgenographically, the mass is frequently mistaken for aneurysm, lymphoma, or some other type of mediastinal tumor.

T. H. NOEHREN

Some Personal Observations and Reflections on the Role of Radiotherapy and Chemotherapy as Primary Treatment for Oat-Cell Carcinoma of the Bronchus. E. K. PILLERS*. *Brit. J. Dis. Chest*, July, 1959, 53: 209–216. (*Addenbrooke's Hosp., Cambridge, England)

All oat-cell carcinomas and other undifferentiated growths of the bronchus should be treated by a combination of chemotherapy and radiation therapy. The present chemotherapeutic drugs are all extremely toxic and, until a better drug is available, it is suggested that nitrogen mustard be used, giving 4 daily doses of 0.1 mg. per kg. during the first 4 days of X-ray treatment. The exact level of dosage of X-ray therapy will be decided by the individual radiotherapist but, in view of the high degree of radiosensitivity of this lesion and the hopeless results of radical therapy, the lower limit of dosage must be contemplated.

It has been shown that "synkavit" (Synkayvite®—sodium menadiol diphosphate) can be used as a radiosensitizer. There was an increase in survival to 10 months from the first treatment in the group of patients treated with intravenous synkavit and X-ray therapy, as opposed to 4.4 months with the X-ray therapy, but in these patients the disease was too advanced for them to tolerate more than about half of a radical level of X-ray treatment. With the lower dose of X-rays and intravenous synkavit, it was possible to demonstrate that a large volume of tissue could be irradiated to a level biologically equivalent to the region of the optimal dose. By this means the therapeutically effective dose was probably increased by about 60 per cent. Clinical trials in this field are being continued at Cambridge and one trial has been designed to show whether oxygen at atmospheric pressure will further increase the effectiveness of synkavit as a radiosensitizer. If further trials with a radioactive synkavit establish its therapeutic effectiveness, this form of treatment may offer considerable advance in the treatment of carcinoma of the bronchus.

M. J. SMALL

Roentgenographic Findings in Primary Pulmonary Carcinoma (in Japanese). H. HOMMA*, K. OTUKI, S. KAGAWA, R. MIKAMI, E. KIRA, and A. YAMANAKA. *Jap. J. Clin. Tuberc.*, October, 1959, 18: 680–688. (*Toranomon Hosp., Tokyo, Japan)

The roentgenographic findings in 185 cases of primary pulmonary carcinoma were analyzed as

to location of primary focus, stadium of the disease, histologic findings, and the specific symptoms of the illness. There were few remarkable findings in the earliest stage of the disease; no specific roentgenographic findings of lung cancer were present, and they were variable during the course of illness. The types of roentgen findings and histopathology had no correlation with each other. The roentgenographic findings were classified into: hilar type (5 triangular or middle lobe syndrome, 8 infiltrative, and 4 tumorous types); mediastinal type (7 bilateral mediastinal, 11 right upper mediastinal, and 3 left upper mediastinal types); and mid-lung field types (18 infiltrative, 12 atelectatic, 15 tumorous, 9 cavitary, 6 multinodular, 11 solitary nodular, 5 miliary, 4 strangular, and 18 hydrothorax types).

I. TATENO

Diaphragmatic Changes Incident to Hepatic Neoplasms. B. EPSTEIN*. *Am. J. Roentgenol.*, July, 1959, 82: 114–119. (*Long Island Jewish Hosp., New Hyde Park, N. Y.)

The roentgenographic diagnosis of liver metastases may be aided by changes in the position, configuration, and range of motion of the right diaphragm. This report describes observations on 25 patients with liver metastases thus identified roentgenographically and confirmed at operation, necropsy, or both. The roentgenographic investigations included fluoroscopic and roentgenographic studies of the chest and roentgenkymographic examinations of the diaphragm. Elevation and impairment of movement of the right diaphragm may be produced by hepatic metastases of sufficient bulk, particularly when the dome of the liver is affected. Such a change is associated with a bulging upward convexity of the right diaphragm and restriction of its motion. Pleural or pulmonary reactive changes are infrequent in these patients.

T. H. NOEHREN

The Effects of Barium Sulfate on the Lungs. A Clinical and Experimental Study. J. WILSON*, P. RUBIN, and T. MCGEE, *Am. J. Roentgenol.*, July, 1959, 82: 84–94. (*Johns Hopkins U. School of Med., Baltimore, Md.)

Both the clinical and experimental observations of this study indicate that barium sulfate acts in the lungs as a relatively inert foreign body. There was no evidence of a toxic reaction in either the group of patients studied or in the experimental animals. Histologic examinations in the animal experiment showed only a mild foreign body reaction to the barium sulfate and no evidence of fibrosis. Both the clinical and experimental investigations showed that the respiratory tract is very efficient in removing barium sulfate from the bronchi.

The experimental investigation did show that it is possible for barium sulfate to produce foci of atelectasis or emphysema by mechanically plugging the bronchi. This possibility is suggested also by one case in the clinical series that showed evidence of emphysematous cysts only in areas where barium sulfate previously had been aspirated. It should be emphasized, however, that these changes, as observed under the conditions of this experiment, were spotty in fashion, affecting only a small fraction of the total lung volume.

From a practical standpoint, it would appear from this study that when aspiration of barium sulfate occurs no reaction need be anticipated and no specific therapy is indicated. Regarding bronchography with barium sulfate, it would appear that there is no contraindication to this procedure, except that instillation of a large amount of barium sulfate into a small volume of the lung may possibly be undesirable. Further study of the mechanical effects of barium sulfate is necessary before any definite conclusions can be drawn.

T. H. NOEHREN

Determination of the Severity of Emphysema by a Roentgenographic Method (in German). G. KALINOWSKI*, A. LICHTERFELD, and F. SPENGLER. *Fortschr. Geb. Röntgenstr.*, January, 1959, 90: 53–61. (*U. of Berlin, West Germany)

Eleven normal and 98 emphysematous patients underwent roentgenographic examinations and various spirometric tests. The roentgenographic examination consisted of a postero-anterior film and lateral inspiration and expiration films. The same techniques and the same synchronized developing process were used for all films. The degree of emphysema was judged by the change in translucency of the retrosternal and retrocardiac spaces during maximal inspiration and expiration. The roentgenographic and spirometric findings were in agreement as to the degree of emphysema in 83.5 per cent of the cases, while in 14.7 per cent there was slight disagreement, and in 1.8 per cent marked disagreement.

H. ABELES

Idiopathic Unilateral Hyperlucent Lung: A Roentgenologic Syndrome. H. MARGOLIN*, L. ROSENBERG, B. FELSON, and G. BAUM. *Am. J. Roentgenol.*, July, 1959, 82: 63–75. (*U. of Cincinnati College of Med., Ohio)

Four cases of a roentgenographic syndrome characterized by unilateral emphysema, a peculiar form of bronchiectasis, and pulmonary

arterial undercirculation are presented. The chief roentgen features include the following: (*1*) abnormal translucency of an entire lung or lobe; (*2*) normal or small size of the affected lung or lobe; (*3*) maintenance of almost constant volume of the affected portion of the lung during changes in respiration; (*4*) a small hilar shadow with sparseness of the intrapulmonary vascular markings; and (*5*) an unusual bronchiectatic pattern without evidence of obstruction of the larger bronchi. The clinical and laboratory aspects of this condition indicate that it is associated with a disability of varying degree, perhaps related to impairment of pulmonary function. Differentiation of this syndrome from other conditions causing abnormal unilateral radiolucency can be made, with bronchography of particular value in this regard. Pulmonary artery hypoplasia presents the most difficult problem in differential diagnosis (after authors' summary).

T. H. NOEHREN

Congenital Deficiencies in the Parietal Pericardium. A Review with 2 New Cases Including Successful Diagnosis by Plain Roentgenography. K. ELLIS*, N. LEEDS, and A. HIMMELSTEIN. *Am. J. Roentgenol.*, July, 1959, 82: 125–137. (*Columbia U. and The Presbyterian Hosp., N. Y., N. Y.)

Two cases are presented of congenital deficiency of the left side of the pericardium. Experience with the first case led to the correct diagnosis of the second case on the basis of the plain chest roentgenograms. In complete absence of the left side of the pericardium, an unusual though apparently typical cardiac silhouette may be present on the plain chest roentgenograms. The heart is shifted toward the left, and the flattened left heart border has an unusually long and well defined pulmonary artery segment. A technique for confirming the diagnosis by pneumothorax is demonstrated. The clinical findings in 97 previously recorded cases of congenital pericardial deficiency are reviewed from the point of view of the roentgenologist. Complete absence of the left side of the pericardium, as in the 2 cases presented here, is the most common type of pericardial defect: it was present in at least 48 of the reported cases.

T. H. NOEHREN

Treatment of Radiation Pneumonitis with Prednisolone. A. C. DOUGLAS*. *Brit. J. Dis. Chest*, October, 1959, 53: 346–355. (*Northern General Hosp., U. of Edinburgh, Scotland)

Four cases of radiation pneumonitis treated with prednisolone are described. Two patients are alive and without respiratory symptoms 2½ years after treatment: 1 has a normal chest roentgenogram, the other has only slight residual pulmonary fibrosis. Two patients died of respiratory insufficiency. Each had shown an initial response to treatment. Deterioration was due to infiltration of the lungs with lymphadenoma in 1 patient, and in the other, uncontrolled, widespread suppurative pneumonia was the immediate cause of death.

There is on record substantial evidence of the failure of steroids either to exert any influence at all on radiation pneumonitis, or to effect more than temporary relief in spite of adequate dosage. It is conceivable that in some cases, perhaps as a result of individual variation in tissue response, a degree of pulmonary damage may occur which precludes reversal of the inflammatory process by corticosteroids. In the absence of definite answers to the many questions which surround the treatment of radiation pneumonitis, it is reasonable to assume that the best results will occur if treatment with corticosteroids is begun early, dosage is reasonably high initially, and treatment is sufficiently prolonged to allow the inflammatory reaction to subside completely. In this last respect it is of interest to note that, in the first 2 cases, the improvement in respiratory function continued for a considerable time after stabilization of the roentgenographic appearances. The latter should not, therefore, be regarded as an index to cessation of treatment which, if premature, may lead to "rebound" phenomena. The most satisfactory response in the present series was observed in a young subject with previously healthy lungs in whom the above conditions of treatment were fulfilled. In this case, complete clearing of roentgenographic opacities and complete restoration of respiratory function were achieved by treatment extending over a period of 18 weeks.

M. J. SMALL

Pulmonary Alveolar Proteinosis. Report of Two Cases. G. LULL, JR.*, J. BEYER, J. MAIER, and D. MORSS. *Am. J. Roentgenol.*, July, 1959, 82: 76–83. (*Fitzsimons Army Hosp., Denver, Colo.)

Two cases of pulmonary alveolar proteinosis have been reported, not only to add to the available case material but to call to the attention of roentgenologists their key role in its identification. The roentgen changes in all cases have been strikingly similar for the same phases of the illness. The characteristic roentgen appearance is said to be that of a fine granular or feathery type of parenchymal density, soft in appearance, usually widely disseminated, and resembling severe pulmonary edema in its radial distribution from the lung hili. This radial distribution was not especially apparent in either of the authors'

cases. Rather, the densities tended to be increased in number and to be more confluent toward the lung bases. In one case it is probable that the changes seen in the left apex are not a feature of pulmonary alveolar proteinosis, but represent a coexistent, unrelated disease process. The histopathologic evidence is that the earliest changes of alveolar proteinosis were making their appearance at the time the specimen was obtained, and they do not correspond with the duration and extent of disease shown on roentgenograms.

The roentgen differential diagnosis could include a wide range of possibilities, but the key (assuming adequate quality of the roentgenograms) is the striking absence of positive physical findings in the presence of extensive changes in the lungs noted on the roentgenograms. There are relatively few other conditions in which this oddity is noted, and the correlation of the clinical information with such roentgen characteristics as the density of the individual lesions, their distribution, associated hilar changes, et cetera, will readily eliminate most from consideration.

The fate of the roentgen pulmonary changes, in common with the histologic changes, has not yet been determined. The pulmonary lesions may remain remarkably unchanged, as was noted in the authors' cases, over periods of months or years, and unrelated to changes in symptomatology. They may also show progression, becoming more extensive in distribution and more confluent. Resolution, when it occurs, is said to begin at the periphery and progress toward the hilus. The points in the differential diagnosis are enumerated. While histopathologic examination is required to confirm the diagnosis, pulmonary alveolar proteinosis may be suspected in a patient who shows extensive roentgen pulmonary changes in the presence of essentially normal findings on physical examination of the chest.

T. H. NOEHREN

Studies on the Accuracy of Diagnostic Procedures. L. GARLAND*. *Am. J. Roentgenol.*, July, 1959, 82: 25–38. (*Stanford U. School of Med., San Francisco, Calif.)

The accuracy of many diagnostic procedures is subject to impairment by errors in technique of examination and interpretation. The former are correctable with care, and the latter partly correctable with training and experience. However, even experienced physicians are found to have a measurable degree of "observer error," due apparently to the so-called human equation. For example, experienced interpreters of a series of plain chest roentgenograms are apt to miss about 30 per cent of those films positive for roentgenographic evidence of a disease, and to over-read about 2 per cent of those negative for disease. Further, in evaluating pairs of serial roentgenograms for alterations in the status of disease, one experienced physician is apt to disagree with another in about ⅓ of the cases, and (on review) to disagree with himself in ⅕ of them.

Accuracy in diagnosis can be improved by independent examination (dual interpretation) either by the same observer on 2 different occasions, or by 2 different observers—without knowledge of the results of the other interpretation. This has been shown to be true for single film examination and for serial film interpretations.

T. H. NOEHREN

Body Section Roentgenography in the Evaluation and Differentiation of Chronic Hypertrophic Emphysema and Asthma. R. FRASER* and D. BATES. *Am. J. Roentgenol.*, July, 1959, 82: 39–62. (*McGill U., Montreal, Canada)

Body section roentgenography was performed on 26 patients with clinical and physiologic diagnoses of hypertrophic pulmonary emphysema. A rapid decrease in caliber and disappearance of the peripheral pulmonary artery branches near their third and fourth stage divisions were regarded as the most important single criterion for diagnosis. The more extreme the arterial tapering, the more evident was the avascularity on the body section roentgenograms. Localization of vessel changes was of some assistance since normally vascularized upper lung fields contrasted sharply with deficient lower lung fields and made appreciation of the latter more obvious. Alteration in caliber of the veins has been noted but has not been of great help in assessment, since its exact significance is not as yet completely understood.

The technique of body section roentgenography as discussed is neither difficult nor time-consuming, and subjects the patient to minimal inconvenience. There is little doubt but that its more extensive use in problems of diagnostic difficulty in chronic lung disease would add considerably to our knowledge of these conditions. The results indicate that, in the roentgenographic diagnosis of pulmonary emphysema, much more precision may be expected from body section roentgenography than from plain roentgenography. The finding that even severe and advanced cases of emphysema cannot, on occasion, be diagnosed from plain roentgenograms of the chest would suggest rather strongly that body section roentgenography should be performed on all patients thought to have emphysema when there is any doubt about the diagnosis. Similarly, the use of laminagraphy in the evaluation of patients with a long history of spasmodic asthma might be expected to reveal

whether any degree of lung destruction has developed that might indicate the presence of early emphysema.

The frequency with which emphysema may occur in a localized form is confirmed by the study of the body section roentgenograms here reported, and certainly suggests that the distribution of the destructive process is often not generalized, even at the stage when considerable respiratory disability exists. The predominant finding of disappearance of vessels in either the upper or the lower lung fields suggests that future investigative work should perhaps be directed at attempts to localize more accurately than is possible at present the main parts of the lung affected.

T. H. NOEHREN

The Normal and Pathologic Bronchographic Pictures of the Glandular Ducts and of Bronchial Diverticula (in German). G. HEIM* and W. HOEFFKEN. *Fortschr. Geb. Röntgenstr.*, June, 1959, 90: 649–656. (*U. of Köln, Germany)

A review of 100 unselected bronchograms, 41 of which had been done because of nonspecific inflammatory processes, revealed the following findings: fine serration on the lower aspect of the main and lobar bronchi in 16 cases, fine serration of the segmental bronchi in 14, larger niche formations in 27, diverticular formation in 1, and no parietal abnormality in 42 cases. The fine serration on the lower aspect of the main and lobar bronchi is the normal bronchographic picture of mucous gland ducts. The serration of peripheral bronchi is abnormal. It is caused either by small ulcerations or dilated gland ducts. Niche formation is due to inflammatory processes. Bronchial diverticula may be congenital, degenerative, or inflammatory in origin.

H. ABELES

Rotational Cinefluorography. F. WINTER* and J. LEHMAN. *Am. J. Roentgenol.*, July, 1959, 82: 120–124. (*Hahnemann Med. College and Hosp., Philadelphia, Penn.)

A procedure is described for axial rotation of experimental animals and human subjects during cinefluorography. The idea of rotary motion of the subject during cinefilming evolved from efforts to obtain a more complete study of coronary vessels by means of multiple projections and a better appreciation of depth relationships. The anatomic displacement of the right and left coronary arteries is sufficient to create a rather striking illusion of their depth relationships when rotation of the subject is employed during coronary arteriography. The application of rotational cinefluorography seemed particularly advantageous to cardiac arrest–coronary arteriography procedures. Acetylcholine-induced cardiac arrest immediately followed by catheter thoracic aortography can effect an excellent 10-second opacification of the coronary arterial system.

At present, definite limitations upon the clinical application of cinefluorography are imposed by presently available equipment and films. The research possibilities of rotational cinefluorography at present outweigh the clinical applications. As techniques and equipment improve to permit study of larger areas, the clinical potentialities will almost certainly broaden. Applications to various anatomic areas, particularly the human heart, may offer far-reaching possibilities for improvement in roentgen diagnosis.

T. H. NOEHREN

Long-Term Follow-up of Children and Adults Exposed to Repeated Fluoroscopic Examinations (1920–1958). A. M. BIRCH* and D. H. BAKER. *A.M.A. J. Dis. Child.*, October, 1959, 98: 530. (*New York Hosp., N. Y., N. Y.)

The recent emphasis on potential hazards of serial fluoroscopic examination for diagnosis has deterred its use in most pediatric centers. This study is concerned with a review of the records of patients and their progeny for whom serial fluoroscopic examinations in the clinic were recorded over the past 38 years. There were approximately 1,500 persons—adults and children—who received an average of 15 to 20 serial fluoroscopic examinations during the period of observation for an average exposure of 10 r each. The incidence of cancer, leukemia, congenital malformations, and stillbirth were found to be no more than would be expected in the general population. It is concluded that no evidence of deleterious effects was obtained in patients or their progeny exposed to serial fluoroscopic examinations during a 38-year period (Authors' summary).

M. J. SMALL

The Roentgenologic Evaluation of Systemic Lupus Erythematosus. T. TAYLOR* and H. OSTRUM. *Am. J. Roentgenol.*, July, 1959, 82: 95–107. (*Philadelphia General Hosp., Penn.)

The roentgen findings in systemic lupus erythematosus depend on the stage of the disease during which the roentgenograms are taken. Changes visible in the chest roentgenograms may suggest the correct diagnosis. These changes are: *Initial acute stage:* Bilateral moderate or massive pleural effusion; massive pericardial effusion; subpleural inflammation; perivascular inflammation; small areas of interstitial pneumonitis at the lung bases; small areas of segmental pneumonitis at the lung bases. *Resolving stage:* Minimal unilateral or bilateral pleural effusion: minimal to

moderate pericardial effusion with straightening of the left border of the cardiac silhouette, but without widening of the heart base; small linear areas of "plate-like" atelectasis at the lung bases; areas of segmental atelectasis at the lung bases. *Chronic stage:* Unless the nephrotic syndrome is present, the cardiac contour returns to normal or cor pulmonale develops. In some instances the outline of the pulmonary conus remains partly obscured by fibrosis which fills in the pericardial spaces around the conus. The pleural effusion may completely disappear. Fine pulmonary scars may be seen at the lung bases, frequently associated with "tenting" of the pleura, or the lungs may appear clear. Multiple pulmonary infections occur. *Exacerbation:* Pleural and pericardial effusions are limited by adhesions; fine linear densities are intermingled with small areas of interstitial pneumonia at the lung bases; basal areas of segmental pneumonitis occur; subpleural and perivascular inflammation are present.

T. H. NOEHREN

The Visualization of Internal Organs by Accentuation Scintillation Scanning Technics. W. J. MACINTYRE*, H. L. FRIEDELL, G. G. CRESPO, and A. M. REJALI. *Radiology*, September, 1959, 73: 329–340. (*Western Reserve U., Cleveland, Ohio)

Two systems have been devised to accentuate and visualize small differences in the uptake of radioactive material by the liver and by various blood pools. The first involves the introduction of a counting rate–controlled cut-off circuit which permits the operator to select arbitrarily a counting rate below which no points are recorded; the second utilizes a light-recording device for accentuating small differences in the counting rate. Examples are shown of the use of these techniques to emphasize the invasion of the liver by both benign and metastatic processes, the separation of pericardial effusion from cardiac enlargement or dilatation, and the differentiation of mediastinal masses from aneurysm of the aorta.

W. J. STEININGER

CHEMOTHERAPY

*The Medical Letter**, January 8, 1960, 2: 1–4. (*A fortnightly non-profit publication on drugs and therapeutics, 136 East 57 St., N. Y., N. Y.)

The following topics are commented upon in this issue: smoking and lung cancer; iron-dextran (Imferon®) in iron-deficiency anemia; pyrvinium pamoate (Povan Suspension®), piperazine (Antepar®), dithiazanine (Delvex®), and tetrachlorethylene in worm infections.

The Medical Letter, January 22, 1960, 2: 1–4.

Chloroquine (Aralen®), hydroxychloroquine (Plaquenil®), and other antimalarial drugs in rheumatoid arthritis and other collagen diseases.

Hydroflumethiazide (Saluron®) and benzydroflumethiazide (Naturetin®) as "thiazide" diuretics in edema or severe hypertension.

Norethynodrel with ethynylestradiol (Enovid®), norethindrone (Norlutin®), and 17-alpha-hydroxyprogesterone caproate (Delalutin®) as synthetic substitutes for progesterone and estrogen.

Disseminated Histoplasmosis Treated with Amphotericin B. R. M. HEYN* and S. T. GIAMMONA. *A. M. A. J. Dis. Child.*, August, 1959, 98: 253–256. (*U. of Michigan Med. School, Ann Arbor)

Disseminated histoplasmosis occurring in infancy is a disease which usually terminates fatally. Only 9 cases of survival in infants have been reported. Various forms of therapy, including supportive measures and the use of such drugs as ethyl vanillate and the diamidine group of compounds, have been used in the treatment of this disease. The course of a 5-month-old infant with disseminated histoplasmosis who was treated with a new fungicidal antimicrobial, amphotericin B, is reported.

The favorable outcome of this infant 1 year after therapy and the negative cultures obtained 3 weeks after treatment was begun are encouraging. Because of the reported toxic effects of amphotericin B, one should carefully observe the patient for any reactions during its use. The reported toxic symptoms include fever, nausea, vomiting, anorexia, and phlebitis at the site of injection. Increased blood urea nitrogen without evidence of renal impairment has been seen when large doses are used. Further use of this drug is justified in the treatment of severe disseminated histoplasmosis.

M. J. SMALL

Experiences with Amphotericin B in Treatment of Histoplasmosis and Moniliasis. H. G. CRAMBLETT*, H. MOFFET, and S. NAJJAR. *A. M. A. J. Dis. Child.*, October, 1959, 98: 509–510. (*University Hosp., Iowa City, Iowa)

The therapeutic efficacy of amphotericin B given intravenously in therapy of fungal diseases of children is unproved and the potential toxicity of the drug is not fully known. This agent was successfully used in treatment of 2 children with disseminated histoplasmosis and a child with monilial infection which were recalcitrant to other means of therapy.

The first patient, a 4-month-old infant, had

disseminated histoplasmosis. Examination of the bone marrow revealed typical *Histoplasma capsulatum*, and cultures of blood and bone marrow yielded growth of this organism. Amphotericin B, 151 mg., was administered in 23 intravenous infusions during a period of 35 days with gradual clinical improvement. At the end of 2 weeks of therapy there was no evidence of the presence of *H. capsulatum* either by culture or examination of the smear. There were no evidences of renal toxicity.

The second patient, a 4-year-old girl with disseminated histoplasmosis, was also successfully treated. A total of 382 mg. of amphotericin B was administered during a period of 42 days by means of 32 intravenous infusions. Fever as high as 38.3°C. (101.9°F.) was noted on 3 occasions in relation to administration of the drug. The concentration of urea nitrogen in the blood was elevated on 5 occasions but became normal when administration of amphotericin B was temporarily discontinued. Hyaline casts were noted in only 1 specimen of urine.

The third patient was a 7-year-old child with moniliasis of 3 years' duration. During the therapy, cultures became negative for *Candida albicans* and have remained so. Transient renal toxicity was observed during the course of therapy.

M. J. SMALL

Antibiotic Sensitivities of Staphylococci Isolated Before and After Patients Were Given Antibiotics. B. A. WAISBREN* and C. L. STRELITZER. *Am. J. M. Sc.*, August, 1959, 238: 202–210. (*Marquette U. College of Med., Milwaukee, Wisc.)

One hundred and fifty-two instances were studied in which the antimicrobial susceptibilities of staphylococci were determined before and after drugs were given to patients. In 53 cases the susceptibility of staphylococci remained the same to the administered antimicrobial, in 57 the second culture was more resistant, in 42 more susceptible. In 17 cases in which a single drug was given, 5 instances of increased resistance were found; in 27 cases where 2 drugs were given simultaneously, 14 instances of increased resistance were observed. Thus, combinations of antimicrobials did not appear to affect the subsequent development of resistance. It is concluded that the administration of an antimicrobial to a patient does not greatly influence the drug susceptibility of staphylococci that are infecting the patient. Subtle influences other than antimicrobial usage must be sought to explain the changing resistances of staphylococci to drugs. Furthermore, the administration of combinations of antimicrobials does not prevent increase in drug resistance of staphylococci isolated from infections.

W. J. STEININGER

Chemoprophylaxis During Respiratory Infections in a Private Pediatric Practice. E. H. TOWNSEND*. *A. M. A. J. Dis. Child.*, October, 1959, 98: 515. (*U. of Rochester School of Med., Rochester, N. Y.)

A study was made of 845 cases of viral respiratory illness occurring during a season when an epidemic of influenza Type A (Asian strain) was present in the community. One of three treatment programs was chosen by a random selection method: (*1*) full therapeutic doses of tetracycline or a sulfonamide, (*2*) prophylactic doses of the same drugs, (*3*) treatment of symptoms. There were no statistical differences among these three treatment groups in duration of illness, complications, or other parameters.

M. J. SMALL

Safe High Dosage Isoniazid Therapy. J. D. P. DAVID* and D. J. LEAHY. *Brit. J. Dis. Chest*, October, 1959, 53: 390–399. (*Wolverhampton and Walsall Hospital Groups, England)

The literature on isoniazid toxicity is reviewed. An investigation into the safety of high-dosage isoniazid treatment was conducted. Between 8 and 16 mg. per kg. of body weight per day was chosen as the most suitable dosage range; a majority of the patients weighed between 8 and 12 stone, and it was found that 600 mg. of isoniazid per day gave this range. Vitamin B-complex tablets were given daily. In those patients whose isoniazid dosage was more than 16 mg. per kg. per day, or who suffered from malnutrition, long-standing tuberculosis, or diabetes, a dose of 50 to 200 mg. of pyridoxine was given daily, depending on the severity of the case.

Three of 90 tuberculous patients developed toxic effects. There are certain types of cases in which high dosage is particularly indicated: patients with tuberculous disease amounting to more than "two zones," and those with one or more cavities, particularly with a "diameter of more than 4 cm.". Another important use of high isoniazid dosage is in the patient who has sputum containing tubercle bacilli partially resistant to isoniazid. It was believed that success was achieved in this way, but the number of cases as yet is too small to make a definite claim. It has been shown that it is reasonably safe to give isoniazid in a higher dosage than has been usual in this country, and it is suggested that a large-scale trial be instituted to investigate fully the value of high-dosage isoniazid treatment.

M. J. SMALL

Use of the Urobilinogen Test for the Detection of Para-Aminosalicylic Acid in the Urine. G. M. LEPEHNE*. *New England J. Med.*, October 29, 1959, 261: 909–910. (*Beth Israel Hosp., Boston, Mass.)

A simple qualitative test for PAS in urine is described which utilizes the appearance of a dark-red color and precipitate in urine after the addition of Ehrlich's reagent. The test should be useful in determining whether therapy is being carried out by the patient (after author's summary).

M. J. SMALL

The Acceptability of Potassium Para-Aminosalicylate. T. MARMION*. *Brit. J. Dis. Chest*, October, 1959, 53: 409–411. (*Killingbeck Hosp., Leeds, England)

During the last year, 30 patients were treated with potassium para-aminosalicylate (KPAS) as part of their combined drug treatment for pulmonary or renal tuberculosis. The first few patients to receive KPAS had gradually increasing doses because of the severe symptoms they experienced with other forms of PAS. When it was realized that the potassium salt was extremely well tolerated, patients were started on full dosage regardless of previous symptom severity. This was 12 gm. daily in 3 doses, the material being made up freshly in water. Patients who have had gastrointestinal symptoms due to the sodium or calcium salt (or derivatives) of PAS do not have these symptoms with the potassium salt. This is remarkably well tolerated, to the extent that it may be considered for the treatment of tuberculous patients with associated or nontuberculous gastrointestinal disease.

M. J. SMALL

Treatment of Acute and Chronic Bronchitis with Depot Sulfonamide (in German). P. H. DOUJAK*, E. F. HUEBER, and H. THALER. *Wien. klin. Wchnschr.*, October 23, 1959, 71: 819–821. (*Medizinische Universitaetsklinik, Vienna, Austria)

A long-acting sulfonamide, sulfamethoxypyridazine (Kynex®), was used for the treatment of bronchitis. The dosage was 2 gm. (2 tablets administered twice) on the first day, and then 1 gm. (1 tablet twice) daily for the treatment of acute bronchitis, and 0.5 gm. (1 tablet) daily for prophylactic administration. Of 26 patients with chronic bronchitis who suffered from febrile bouts of putrid bronchitis, the acute complication was improved in 21 (80 per cent)—in some of these when broncholytic inhalations were given simultaneously. Of 21 patients with chronic bronchitis who were given prophylactic treatment during the winter months, no febrile relapses occurred in 9 (43 per cent). Of 55 patients treated with this medication, 4 developed allergic reactions; 19 complained of dryness of the mucosa of the nose, mouth, and pharynx.

G. C. LEINER

PULMONARY PHYSIOLOGY

A Bronchospirometric Study on Impairment of the Respiratory Reserves Caused by Parenchymal and Pleural Changes (in Finnish). V. AUTIO*. *Duodecim*, August, 1959, 75: 536–550. (* Preitilä, Finland)

Bronchospirometry was performed on 132 patients with unilateral tuberculous lesions. Parenchymal changes caused a small, and pleural changes a considerable, loss of pulmonary function. Oxygen consumption was the entity most involved in the diseased lung, vital capacity in a lesser degree, and ventilation least of all. Especially in the presence of pleural changes, a relative hyperventilation was present: i.e., in the involved lung the ventilation was much less impaired than the oxygen consumption.

J. HAAPANEN

The Use of Mechanical Assistance in Treating Cardiopulmonary Diseases. F. D. GRAY, JR.* and A. S. FIELD, JR. *Am. J. M. Sc.*, August, 1959, 238: 146–152. (* Yale U. School of Med., New Haven, Conn.)

A rhythmically inflated abdominal belt functioning alternately with mouth inspiratory positive pressure has proved to be an effective method for ventilating patients with severe pulmonary emphysema. It helps reduce overdistention, lowers alveolar carbon dioxide tension, and maintains blood pressure in cases of vasomotor instability. An apparatus providing alternate and rhythmic electric neuromuscular stimulation of the diaphragm and lower abdominal muscles also provides adequate ventilation in emphysematous patients. It shows promise as an instrument useful in initiating diaphragmatic breathing exercises in those patients whose disease has impaired diaphragmatic motion (Authors' summary).

W. J. STEININGER

Intermittent Positive Pressure Breathing: An Evaluation of Its Use in Aerosol Therapy of Chronic Pulmonary Emphysema. J. T. TAGUCHI*. *Am. J. M. Sc.*, August, 1959, 238: 153–161. (* V. A. Center, Dayton, Ohio)

In a study of the effects on ventilatory function of pressure-nebulized bronchodilators administered with and without intermittent inspiratory positive pressure breathing (IPPB/I) in 29 patients with pulmonary emphysema, it was clearly evident that the addition of IPPB/I produced no

significant difference in the degree of improvement. The patients' choices as to the most effective method could not be correlated with objective measurements. In a second study, changes in ventilatory function following pressure nebulization of a placebo with and without IPPB/I were compared in 25 patients with pulmonary emphysema. No significant beneficial or harmful effects on ventilatory function attributable to IPPB/I were shown.

Contraindications to IPPB/I are few and include recent pneumothorax, pulmonary hemorrhage, shock, and certain cases of decreased cardiovascular reserve. Preliminary studies reveal decreases in oxygen saturation following IPPB/I/Air in a few patients. From a practical standpoint, the automatic and intermittent nebulizing features of IPPB/I which save oxygen and aerosol make this apparatus extremely useful. By using mask pressure below 5 cm. of water, except when specifically indicated, and employing oxygen as the pressure source, this unit has proved to be safe, effective, and convenient for routine aerosol treatment. Emphasis, however, is placed on the need for individualizing treatment.

W. J. STEININGER

Greater Efficiency in Oxygen Therapy: An Improved Oxygen Tent. E. J. BREWER, JR.* and D. SINGER. *J. Pediat.*, October, 1959, 55: 504–510. (* Baylor U., Houston, Texas)

The standard open-bottom oxygen canopy in routine hospital use does not provide adequate concentrations of oxygen. An efficient oxygen canopy is described which embodies several well-known principles of oxygen therapy. The essential features of the tent are: a closed bottom to prevent leakage of oxygen, a canopy which covers only the head to permit more frequent examinations and procedures, a smaller canopy which requires less oxygen to obtain optimal concentration, an open top to minimize patient apprehension and allow easy access to the patient's face, and a detachable top which can be employed in the event that strong air currents occur in the room.

In every hospital, frequent determinations of oxygen concentrations should be made routinely in all types of canopies to assure the anoxic patient continuous and adequate oxygen. The canopy described is particularly useful for patients 4 years of age and older, including adults. Patient acceptance has been good. This canopy provides adequate, economical, and practical oxygen therapy to the patient in the usual hospital situation.

M. J. SMALL

MICROBIOLOGY AND IMMUNOLOGY

Neuraminidase-Like Factors in Cultures of Pneumococci, Alpha-Streptococci and Pasteurella Pseudotuberculosis. A. LAURELL*. *Acta path. et microbiol. scandinav.*, No. 2, 1959, 47: 182–190. (* Bacteriological Institute, U. of Lund, Sweden)

Incubation of human serum with pneumococci, some alpha-streptococci, *Pasteurella pseudotuberculosis*, and *Clostridium perfringens* produced a pronounced change in the electrophoretic pattern in the alpha-beta region. The pattern then resembled that seen on incubation of serum with cholera filtrate. A fair portion of the protein-bound sialic acid in serum was split off by cultures of pneumococcus type XXXI and *Pasteurella pseudotuberculosis*. The alpha-streptococci tested also split off the protein-bound sialic acid. Incubation of human erythrocytes at 37°C. with a sterile filtrate of cultures of pneumococcus type XXXI destroyed the virus receptors, and the cells showed the Thomsen-Friedenreich phenomenon (agglutination occurred in AB-serum). Incubation of the erythrocytes with sterile filtrates of cultures of some alpha-streptococci produced the viral type of hemagglutination and the Thomsen-Friedenreich phenomenon.

E. DUNNER

Diagnosis of Pneumococci by the Optochin and Bile Tests. E. LUND*. *Acta path. et microbiol. scandinav.*, No. 3, 1959, 47: 308–315. (* State Serum Institute, Copenhagen, Denmark)

The identification of pneumococci, smooth (S) and rough (R), may be made by the hydrochloride of ethylhydrocupreine (Optochin®) test, which is performed in practice with Optochin tablets (6 mm.-0.05 mg.) or Optochin discs (6 mm. moistened in 0.05 per cent solution of Optochin in distilled water) when inhibition zones of > 20 mm. indicate a pneumococcus, while streptococci generally give no zone or, at any rate, smaller zones (< 15 mm). The Optochin test is more reliable than the bile test in differentiating between pneumococci and streptococci.

E. DUNNER

Immunochemical Studies of Soluble Antigens of *M. Tuberculosis*. Immunochemical Study of Two Polysaccharides Present in the Filtrates of Tubercle Bacilli. P. BURTIN*. *Ann. Inst. Pasteur*, September, 1959, 97: 325–339. (* Saint Antoine Hosp., Paris, France)

An immunochemical relationship exists between the polysaccharides I and III present in tubercle bacilli and culture filtrates. This relationship can be demonstrated with immune serum, which may react with only 1 of the 2 polysaccharides, be

totally absorbed by 1 polysaccharide and partially absorbed by another, or be only partially absorbed by each of the 2 polysaccharides. These different reactions depend on the qualitative constitution of the immune serum. The immunochemical relationship of the 2 polysaccharides is due to a partial identity of their constitution, the molecule of both containing mannose and arabinose. Inhibition experiments have proved that these sugars were at least partly responsible for cross reactions.

V. LEITES

Are Slide Cultures Suitable for the Rapid Culture of *M. Tuberculosis*? (in German). E. JUNGFER* and B. SCHNEEWEISS. *Tuberkulosearzt*, August, 1959, 13: 537–545. (* Clara-Zetkin-Str. 96, Berlin W 8, Germany)

The slide-culture method was found to be inadequate for culturing *M. tuberculosis* for the following reasons: positive results were obtained only if the material used for inoculation contained a large number of bacilli; the results were often unreliable; the persons handling the slides are exposed to a serious risk of infection. This technique is therefore not recommended for routine bacteriologic work.

J. HAAPANEN

Complexity of the Mixture of Fatty Acids from Tubercle Bacillus. Acids with Less than Twenty Carbon Atoms. C. L. AGRE* and J. CASON. *J. Biol. Chem.*, October, 1959, 234: 2555–2559. (* U. of California, Berkeley)

Esters of acids in the C_{14} to C_{19} range from several lots of tubercle bacilli (H37Rv and H37Ra strains) were anlayzed by gas chromatography. The pattern of acids in the several lots of lipids was found to be similar qualitatively, but to differ quantitatively. Eleven acids were detected: ⅓ of the total distillable acids were palmitic acid, and an additional ⅓ were at the C_{18} and C_{19} range. 10-Methyloctadecanoic acid (tuberculostearic) and 10-methylheptadecanoic acid were also present.

E. SOTO LEAKE

Early Detection of Microcolonies of Tubercle Bacilli (in Japanese). T. ITO*, H. OKAWA, and I. SUGIYAMA. *Clin. Resp. Organs*, October, 1959, 14: 813–818. (* National Kanagawa Sanatorium, Kanagawa Prefecture, Japan)

The authors describe a method of detecting microcolonies of tubercle bacilli as early as 10 to 12 days following inoculation of NaOH-treated sputum to "3 per cent Ogawa medium." A filter paper 35 mm. by 16 mm. in size is placed on the slant of the medium, and the digested sputum is run down over it. After incubation for 10 to 12 days the filter paper is removed, placed on slide, fixed with diluted ovalbumin solution, and stained. The slide is microscopically observed after removal of the paper. The medium from which the filter paper was removed is further incubated. Of 444 sputum specimens, 144 or 79 per cent of the cultures were positive for tubercle bacilli by this method on the 12th day of inoculation (visible colonies naturally developed on further incubation in these 144 cases); and 38 other samples which were negative by this method later turned positive, as demonstrated by the development of colonies on the slant from which filter paper had been removed. In short, 182 samples were positive by this method while 187 samples were positive by the ordinary method of cultivation with the same medium.

I. TATENO

Studies on the Peroxidase and Catalase Activities of Freshly Isolated Tubercle Bacilli (in German). R. VON BERTRAB*. *Schweiz. med. Wchnschr.*, August 15, 1959, 89: 868–870. (* U. of Basel, Switzerland)

Qualitative enzyme determination was done on 99 strains of *M. tuberculosis* using the pyrocatechol test as described by Vischer and Tirunarayanan. Of 21 isoniazid-resistant strains, 18 showed catalase activity, but in 15 strains no peroxidase activity was found. A close correlation between the diminished or absent peroxidase activity and the isoniazid resistance could be demonstrated on subcultures in Youmans fluid medium. On the other hand, 8 of 78 isoniazid-susceptible strains had negative peroxidase reactions, possibly due to the onset of resistance. The differences between the direct culture and the subculture (on Löwenstein and Youmans medium, respectively) are interpreted as the influence of an undetermined medium factor. It is concluded that the directly isolated strains represent predominantly mixed populations composed of susceptible and resistant germs, and that the pyrocatechol test is more specific for the demonstration of a mixed population than is the catalase reaction alone.

J. HAAPANEN

Study of Intrinsic Factors of *M. Tuberculosis* Explaining the Quiescent State and the Lack of Growth on Usual Culture Media (in French). P. J. COLETSOS*. *Ann. Inst. Pasteur*, September, 1959, 97: 290–306. (* Pasteur Institute, Paris, France)

The survival and persistence of *M. tuberculosis*, although seriously challenged by chemotherapy, is nevertheless assured by the more or less pro-

longed survival of "sensitive persistent" elements within the tuberculous organism and the passing of certain other elements into a "quiescent" state. Sensitive persistent elements and even more quiescent bacilli can be demonstrated *in vitro* only in the presence of organic stimulating substances, very similar to those present in the human body. Substances extracted from monkey organs possess an almost specific stimulating effect on the growth of such bacilli *in vitro*. This fact might account for relapses and persistence of active lesions in tuberculous patients who excrete apparently nonviable bacilli, and would confirm the hypothesis that certain pathogenic bacilli might find conditions in the human body which are absolutely necessary for their development and which do not exist on the usual culture media or in guinea pigs. A correlation may exist between the described experimental data and the easy transmission from man to man of these bacilli endowed with such exceptional biologic requirements. If this is true, one could assume that *M. tuberculosis*, due to antimicrobial therapy, undergoes an involution which tends to liken it to the Hansen bacillus (after author's summary).

V. LEITES

EXPERIMENTAL PATHOLOGY

Influence of Cortisone or Corticotropin on Experimental Aspergillosis in Mice (in Japanese). H. SUGIMOTO*. *Nisshin Igaku*, November, 1959, 46: 675–686. (* Hosp. of the Institute for Infectious Diseases, U. of Tokyo, Japan)

The influence of these hormones was studied on mice experimentally infected with small (10^2–10^3) or large (10^6–10^7) numbers of *A. fumigatus* or *A. niger* spores. Continuous administration of cortisone or corticotropin revealed definite enhancing effects on the establishment of chronic mycetoma of *A. fumigatus* in the primary inoculation sites of the subcutaneous connective tissue or the peritoneal surface. When a single large dose of cortisone was given simultaneously with the fungal inoculum, development of mycelium started within a few hours after the inoculation of a small number of spores, while hematogenous dissemination became more severe upon infection with a large number of spores.

Aspergillus niger was pathogenic for and killed mice only by the intravenous route, while the lesions were localized and no mycelial growths developed by other routes of inoculation; administration of cortisone or corticotropin accelerated the fatal infection in the former, and caused hematogenous infection in the latter case, respectively.

I. TATENO

Less Well Known Properties of Antituberculosis Drugs (The "M Substances") (in German). P. MASCHERPA*. *Wien. klin. Wchnschr.*, October 23, 1959, 71: 826–829. (* U. of Pavia, Italy)

"Organotropism" is the more or less selective localization of drugs in certain organs: streptomycin and PAS are concentrated in the lungs to a high degree; isoniazid is concentrated in the kidneys, liver, and lungs. Isoniazid and PAS decrease the oxygen utilization of the tissue, as shown by the Warburg method. Isoniazid, pyrazinamide, and streptomycin decrease the activity of the cocarboxylase. A complex of substances with antituberculosis activity was found in various tissues of mammals (including humans), especially in the lungs. This is called substance "M," and is composed of substances M_1 and M_2. M_1 has been given with beneficial results to tuberculous patients by subcutaneous injections.

G. C. LEINER

Experimental Pulmonary *Pneumocystis Carinii* Infection in Rabbits. W. H. SHELDON*. *J. Exper. Med.*, July, 1959, 110: 147–160. (* Emory U. School of Med., Atlanta, Georgia)

Pneumocystis carinii (interstitial plasma cell pneumonia) is a common human disease in Europe, with its highest incidence among debilitated infants 6 weeks to 4 months of age. The disease is contagious and is characterized by progressive respiratory failure, with a mortality rate of 25 per cent. The histologic findings of large numbers of *Pneumocystis carinii* organisms in the interstices of alveolar tissue suggest a causal relationship of this organism to the disease. A different form of the illness has been reported previously from this laboratory in which *Pneumocystis carinii* was an incidental finding at autopsy of 4 patients, with no clinical manifestations produced by the organism. Following the study of another patient with widespread Pneumocystis pneumonia, the present report describes attempts to reproduce the disease in rabbits.

(*1*) Young male and female rabbits were inoculated with either infected human or rabbit lung tissue and were treated for various periods with cortisone and antimicrobials or with the latter alone. (*2*) Controls were similarly treated, but were inoculated with either saline or boiled normal human lung tissue. (*3*) Another group of animals, untreated and noninoculated, were examined for latent Pneumocystis infection. Marked interstitial pneumonitis with many Pneumocystis organisms were found in animals from groups (*1*) and (*2*). Minute foci of pneumonitis and only rare numbers of organisms were seen in animals of group (*3*). These findings do not establish the

transmission of the disease from human beings to rabbits, but they indicate that latent pulmonary Pneumocystis infection is widespread among the animals tested and is activated by an impairment of host resistance. A series of photomicrographs presents the morphologic characteristics of *Pneumocystis carinii* and the histopathology of infected lung tissue.

C. H. PIERCE

Studies on Tubercle Bacillus–Monocyte Relationship. III. Conditions Affecting the Action of Serum and Cells. Modification of Bacilli in an Immune System. J. FONG*, D. CHIN, H. J. AKIYAMA, and S. S. ELBERG. *J. Exper. Med.*, June, 1959, 109: 523–543. (* U. of California, Berkeley)

Experiments designed to observe the behavior of virulent tubercle bacilli (H37Rv) in the presence of normal or immune rabbit cells and serum were carried out in Mackaness-type culture chambers to study degeneration of the host cells, and in Carrel flasks to determine bacterial multiplication. Normal adult rabbits or rabbits injected intradermally with living or heat-killed BCG were used as monocyte (peritoneal exudates) and serum donors. Infecting inocula of tubercle bacilli were obtained from bacterial cultures grown 7 days in Tween®-albumin medium.

When cultivated in normal serum, the cell population of infected immune cells decreased markedly compared to similarly infected cells grown in the presence of anti-BCG or anti-Salmonella serum. This protective serum effect was not destroyed by dialysis or by heating to 70°C. The activity remained after absorption of the serum with homologous antigens, thus demonstrating no close association with antibody globulin. Moreover, parallel experiments with serum globulin fractions failed to show a protective effect from the use of immune globulin. The protective serum factor could be elicited when cells and serum were obtained from rabbits as early as 5 days after immunization with BCG—at a time when the animals gave a negative tuberculin response.

The capacity of virulent tubercle bacilli to induce degeneration of monocytes, or their ability to multiply intracellularly, was not affected by pretreatment of the bacteria with immune serum. Moreover, the cultivation of normal and immune monocytes in normal or immune serum had no influence upon the native susceptibility or resistance of these cells. However, modification of the bacterial cells was observed when tubercle bacilli were cultured in the presence of immune monocytes and immune serum before being used as infective inocula. Tubercle bacilli obtained from this system failed to cause degeneration of normal monocytes cultured in immune serum.

C. H. PIERCE

Prevention of the Development of Tuberculosis with Antimicrobials. Influence of Prior BCG Vaccination on the Outcome (in Japanese). F. SHIMADA*. *Kekkaku*, October, 1959, 34: 699–704. (*School of Med., Nagoya U., Japan)

Experiments were made in 80 guinea pigs. They were infected with streptomycin-susceptible H37Rv tubercle bacilli irrespective of whether the animals had been BCG vaccinated. Animals vaccinated with BCG were untreated, or were treated with isoniazid; unvaccinated animals were treated with isoniazid. Histopathology was remarkably less pronounced in these three groups—especially in the second group—than in the control animals. The first and third groups did not show essentially different degrees of changes from each other.

The same results were obtained when the animals were infected with streptomycin-resistant H37Rv tubercle bacilli, although the tuberculous lesions were less remarkable in the unvaccinated isoniazid-treated group than in the BCG-vaccinated untreated group. Development of tuberculin allergy was minimal in the animals treated with isoniazid irrespective of prior vaccination with BCG.

I. TATENO

Protection Against Tuberculous Superinfection (in German). E. FREERKSEN*. *Deutsche med. Wchnschr.*, August 28, 1959, 84: 1533–1540; and September 4, 1959, 84: 1617–1619. (* Tuberkulose-Forschungsinstitut, Borstel/Bad Oldesloe, Germany)

Survival experiments with mice, guinea pigs, and rabbits showed that animals infected with minimal doses were able to survive tuberculous superinfection longer than those not so infected. The protective effect against superinfection was maximal 2 to 6 weeks after the initial infection, depending on the bacterial strain used. Saprophytic or apathogenic mycobacteria had no protective effect, while avirulent variants of pathogenic tubercle bacilli (BCG and H37Ra) provided good protection against superinfection with virulent organisms. In mice, a subcutaneous vaccination with virulent organisms provided a definite protection against intravenous superinfection with the same virulent strain. This effect was not demonstrable in guinea pigs and rabbits, in which subcutaneous injection of virulent bacteria led to a rapidly progressing tuberculosis. Protection against superinfection with a virulent strain of tubercle bacillus was thus achieved only with a pathogenic form. Mycobacteria were designated

"pathogenic" if they caused typical tuberculous foci in the infected organism. This capacity was preserved even when they were killed. The "pathogenicity factor" probably resides within the phosphatide fraction of the tubercle bacillus.

The development of tuberculosis as a disease requires not only pathogenicity of the microorganism, but also that it be capable of multiplying in the organism, which characteristic is called "virulence." Apparent virulence can be conferred on killed pathogenic microorganisms by adjuvants which secure prolonged contact between the "pathogenicity factor" and the tissue. "Generalization" may then produce tuberculosis without living microorganisms. The virulence, however, is determined not only by the properties of the microorganism, but also by the properties and conditions of the host organism: e.g., BCG is virulent for the golden hamster. Changes in these factors are responsible for the activation of a previously inactive tuberculosis. These changes may even lead to BCG tuberculosis in man.

The avian tubercle bacillus conferred a protection against tuberculous superinfection which was half-way between the saprophytes and human tubercle bacilli. The scotochromogenic, photochromogenic, or otherwise atypical strains isolated from human lesions behave similar to the avian variant. Protection against superinfection was achieved even with killed bacteria if the vaccination dose was high and the infecting dose low. The protective effect of the killed bacteria was augmented by adjuvants.

It has so far proved impossible to assign the protective effect to any one fraction or component of the tubercle bacillus, or to establish a definite causal relationship between allergizing effect and protection against superinfection. It is thus necessary to distinguish allergy from immunity. Tissue-culture experiments and other studies indicate that a prior infection acts largely by improving the organism's natural protective mechanisms. It is not simply an immunologic phenomenon, but a part of the biologic course of the infection. The described experiments showed that the value of BCG vaccination can be deduced not only from epidemiologic data, but also from animal experiments.

J. HAAPANEN

The Effects of Ultracortenol® on Experimental Tuberculosis (in German). M. JANOWIEC* and J. VENULET. *Tuberkulosearzt*, August, 1959, 13: 560–563. (* Pharmacologic Institute, Warsaw, Poland)

Mice and guinea pigs were infected with *M. tuberculosis* after half of them had been inoculated with BCG. They were then treated with isoniazid and/or prednisolone trimethyl acetate (Ultracortenol®). These 2 drugs seemed to have synergistic action. If given singly, neither had any effect on the course of tuberculosis. The simultaneous administration of isoniazid and Ultracortenol to BCG-immunized guinea pigs resulted in prolongation of life and reduction of the mortality rate as compared to the control group. The protective effect was absent in nonimmunized guinea pigs. Because Ultracortenol has very few side effects, it might be preferable for tuberculous patients requiring prolonged treatment.

J. HAAPANEN

Anaphylactic Shock in Mouse. II. Allergenicity of Water-in-Oil Emulsions With or Without Mycobacterium. P. MORGAN*, A. A. WERDER, R. SODERBERG, and N. P. SHERWOOD. *Proc. Soc. Exper. Biol. & Med.*, October, 1959, 102: 161–163. (* U. of Kansas, Kansas City)

Mice sensitized with 0.1 mg. of bovine albumin incorporated into water-in-oil emulsion containing *M. butyricum* showed anaphylactic reactivity when challenged 28 days postsentitization with as little as 0.01 mg. of bovine albumin. Signs of anaphylaxis were observed 196 days after sensitization. Similar results were obtained with bovine albumin in emulsions lacking *M. butyricum*.

E. SOTO LEAKE

Hemorrhagic Reactions in Animals Sensitized with Ovalbumin in Adjuvants Containing Mycobacteria. II. Relation to "Delayed" Hypersensitivity. S. H. STONE* and J. FREUND. *Proc. Soc. Exper. Biol. & Med.*, October, 1959, 102: 140–146. Dept. of Health, Ed., & Welfare, Bethesda, Md.)

The subcutaneous injection of egg albumin in guinea pigs sensitized with the same antigen incorporated in water-in-oil adjuvant containing *M. tuberculosis* elicited massive hemorrhages in the skin and subcutaneous tissue. This reaction failed to occur in guinea pigs which were sensitized with egg albumin at one site and mycobacteria in oil at a remote site.

E. SOTO LEAKE

Survival and Multiplication of BCG and *M. Tuberculosis* in Mice as Related to Time. A Method of Determination (in French). M. PANISSET* and J. C. BENOIT. *Rev. tuberc., Paris*, October, 1959, 97: 437–465. (*U. of Montreal, Canada)

BCG strains do not produce any progressive pulmonary lesions in mice but, after a certain

delay, they often cause severe, sometimes fatal, caseous renal lesions. Isoniazid-resistant strains, even if avirulent for guinea pigs, always cause rapidly progressive pulmonary lesions which kill mice without causing renal lesions. Among atypical strains, the photochromogenic strains—all more or less virulent for mice—produce fatal pulmonary and renal lesions. Scotochromogenic mycobacteria do not produce death in mice, and cause neither renal nor pulmonary lesions. However, in contrast to saprophytic mycobacteria (which belong to this group), bacilli persist and grow in the organs without causing lesions. Culture of organs reveal the presence of very numerous bacilli. Nonphotochromogenic mycobacteria, of which only 2 strains were studied, have varying pathogenicity. One was virulent for mice, causing severe renal lesions; the other produced only minimal pulmonary foci. *Mycobacterium balnei* is easy to differentiate from the other mycobacteria by the lesions which it produces in the tail and legs of mice.

V. LEITES

UPPER RESPIRATORY TRACT DISEASE, BACTERIAL

Streptococcal and Non-Streptococcal Sore Throat. V. ESMANN*. *Acta med. scandinav.*, March 23, 1959, 163: 265–276. (* Marselisborg Hosp., Aarhus, Denmark)

Of 81 patients with sore throat, this symptom was due to streptococci in 42 per cent and to adenovirus in 5 per cent, while 7 per cent had mononucleosis, 2 per cent Vincent's disease, and 44 per cent had to be classified as "undifferentiated acute respiratory diseases." As streptococcal and nonstreptococcal sore throat cannot be differentiated on the basis of clinical observations, routine cultures of pharyngeal material for the isolation of B-hemolytic streptococci are indicated in patients with sore throat. An increase in antistreptolysin titer (AST) was found to be a significant sign of streptococcal sore throat when this increase amounted to 100 per cent of the initial AST and when the convalescent-phase titer exceeded 100. An increase in the antistreptococcal-hyaluronidase titer (ASH) was found to be a significant sign of streptococcal sore throat when the convalescent-phase titer exceeded 9,000. A significant increase in one or both antibodies was revealed in 62 per cent of the patients.

E. DUNNER

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1. Cohen, R. V., Lambert, R. L., and Molthan, L.: The Use of POTABA in Sarcoidosis; Annual Meeting American Trudeau Society, Eastern Section, Boston, Mass. (1958).
2. Zarafonetis, C. J. D., and Horrax, T. M.: Treatment of Peyronie's Disease with POTABA, Journal of Urology, 81: 770-772 (June 1959).

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Write for samples, literature, complete information

THE Panray CORP.

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